

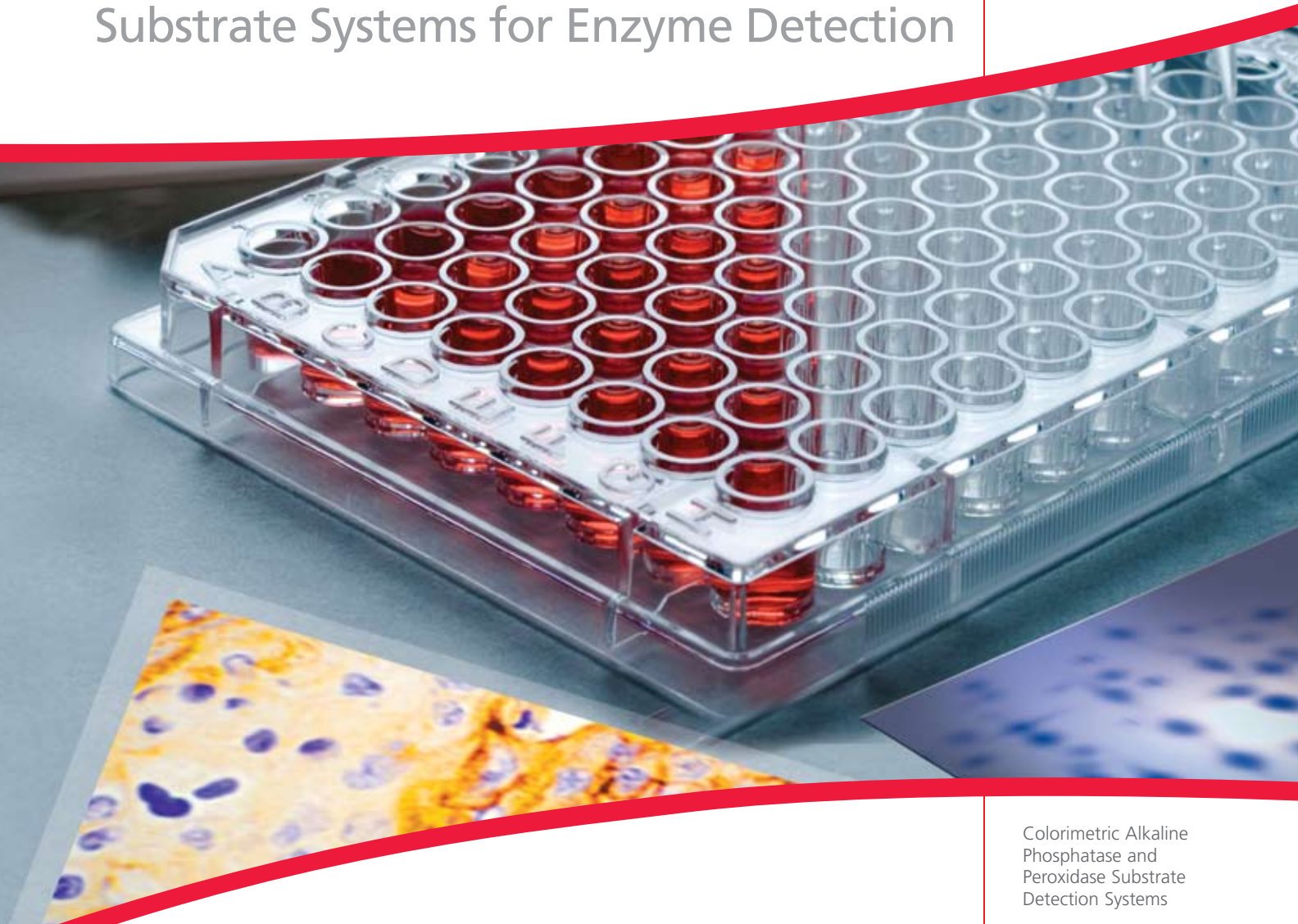
Life Science

# BIOFILES

Volume 3, Number 4



## Substrate Systems for Enzyme Detection



Convenient detection systems for:  
*Immunohistology*  
*Western Blotting*  
*ELISA*

Colorimetric Alkaline  
Phosphatase and  
Peroxidase Substrate  
Detection Systems

Luminescent Alkaline  
Phosphatase and  
Peroxidase Substrate  
Detection Systems

Other Substrate  
Detection Systems

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## Enzyme Explorer—Substrate Index

The Enzyme Explorer is a comprehensive web resource for researchers working with enzymes/proteins, substrates, activators, or inhibitors. With nearly one thousand substrate products, the Enzyme Explorer's Substrate Index provides an easy-to-use interface for locating substrates.

**Use the Substrate Index to locate substrates by:**

- Application
- Enzyme
- Detection Method

[sigma.com/enzymeexplorer](http://sigma.com/enzymeexplorer)



## Antibody Explorer—Substrates, Buffers, and Blockers

In addition to the Enzyme Explorer's Substrate Index, the Antibody Explorer is the direct portal to substrates and reagents for antibody detection applications.



**Find substrates and reagents for:**

- Western blotting
- ELISA
- Immunohistology
- Antibody labeling

[sigma.com/antibodyexplorer](http://sigma.com/antibodyexplorer)

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# Introduction



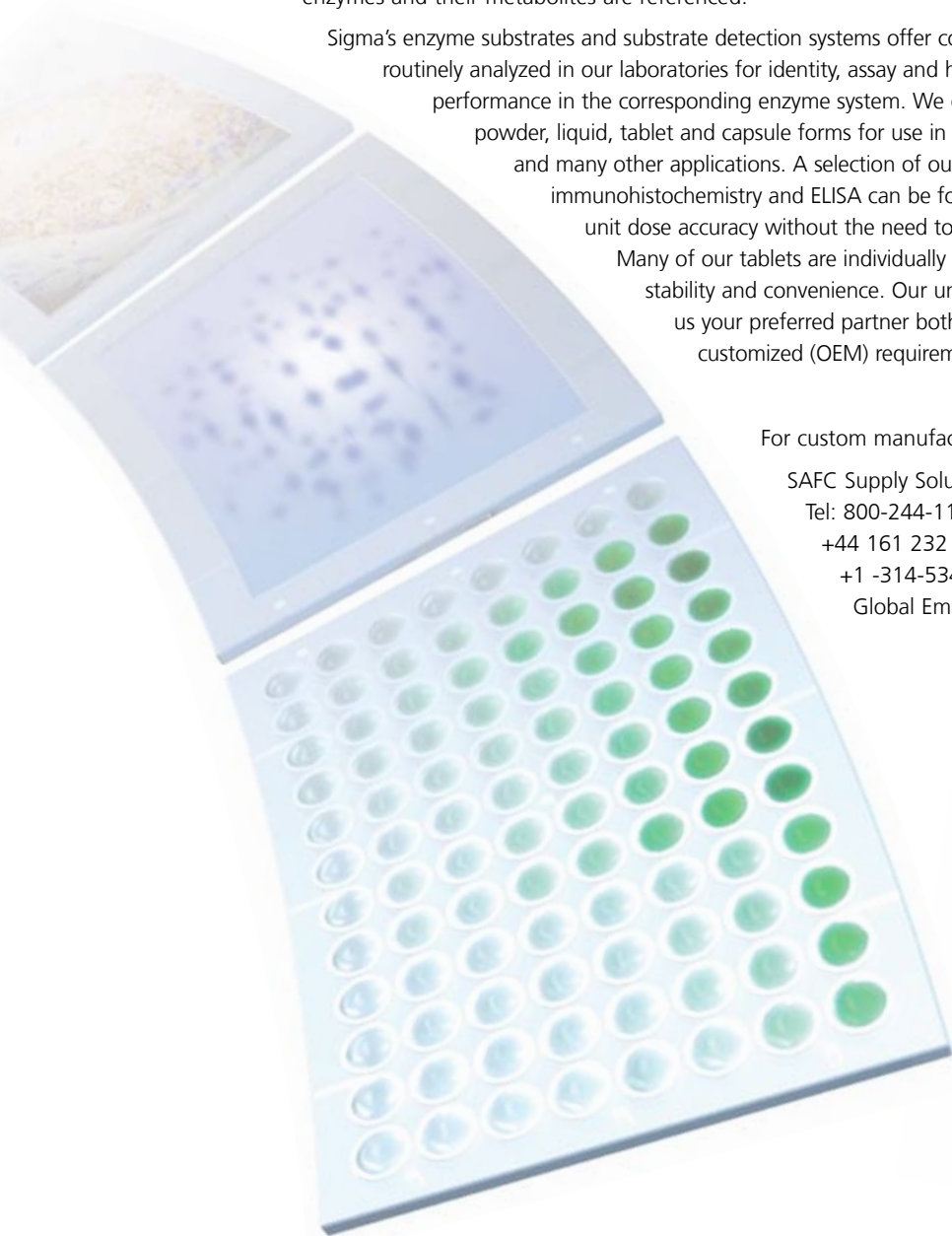
Roland Wohlgemuth  
[roland.wohlgemuth@sial.com](mailto:roland.wohlgemuth@sial.com)

Substrates continue to be of prime importance in many areas of the life sciences, both as tools and targets in detection systems. Substrates for the detection of enzyme activities are presented in this BioFile. Additional enzyme kits and systems for the analysis of substrates as targets are detailed on the Enzyme Explorer. The Enzyme Explorer Assay Library also shows how the wealth of natural and synthetic substrates can be approached from the different enzyme activities to be detected. Another entry to the natural substrates can be found on our website [sigma-aldrich.com/metabolomics](http://sigma-aldrich.com/metabolomics), where the metabolic enzymes and their metabolites are referenced.

Sigma's enzyme substrates and substrate detection systems offer convenience, stability, consistency and are routinely analyzed in our laboratories for identity, assay and homogeneity, as well as for functional performance in the corresponding enzyme system. We offer substrates, buffers and reagents in powder, liquid, tablet and capsule forms for use in enzyme assays, ELISA, blotting, imaging and many other applications. A selection of our enzyme substrates for western blotting, immunohistochemistry and ELISA can be found in tables 1-3. Our tablets offer unit dose accuracy without the need to weigh or handle hazardous materials. Many of our tablets are individually packaged in foil packets for enhanced stability and convenience. Our unrivalled manufacturing experience makes us your preferred partner both for your research and large-scale and customized (OEM) requirements.

For custom manufacturing inquiries:

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+44 161 232 5500 (Europe)  
+1 -314-534-4900 (International)  
Global Email: [safcglobal@sial.com](mailto:safcglobal@sial.com)



## Western Blotting

Substrate Composition	Liquid Substrate Systems		Tablets		End Product	Detection/Color
	Prod. No.	Product Description	Prod. No.	Product Description		
<b>ALKALINE PHOSPHATASE</b>						
BCIP/NBT	B1911	BCIP/NBT Liquid Substrate System, Tru-Measure	B5655	SIGMA FAST™ BCIP/NBT System (10 mL)	Insoluble	Blue/Purple
	B3679	BCIP/NBT-Purple Liquid Substrate for Membranes	B0274	BCIP Tablet (25 mg)		Purple
	B3804	BCIP/NBT-Blue Liquid Substrate for Membranes	N5514	Nitro Blue Tetrazolium (10 mg)		Blue
BCIP Analog/INTX	AR0300	Alkaline Phosphatase Red Membrane Substrate			Insoluble	Red
	AR0400	Alkaline Phosphatase Red Membrane Substrate				
BCIP Analog/NBT	AB0300	Alkaline Phosphatase Blue Membrane Substrate			Insoluble	Blue
	AB0400	Alkaline Phosphatase Blue Membrane Substrate				
Chemiluminescent	C0712	CDP-Star				
Fast Red/Naphthol AS-MX			F4648	SIGMA FAST Fast Red TR/Naphthol AS-MX Tablets (1 mL)	Insoluble	Red
			F4523	SIGMA FAST Fast Red TR/Naphthol AS-MX Tablets (10 mL)		
			F5146	Fast Red RC Tablet (10 mg)		
			N8518	Naphthol AS-TR phosphate disodium salt (4 mg)		
(TAC) Buffer/ Hexazonium Initiator/Magenta	AM0100	Alkaline Phosphatase Magenta Substrate			Insoluble	Magenta
<b>PEROXIDASE</b>						
AEC			A6926	3-Amino-9-ethylcarbazole (20 mg)	Soluble	Brown
Chemiluminescent	CPS1-60	Chemiluminescent Peroxidase Substrate (60 mL)			Soluble	Chemiluminescent
	CPS1-120	Chemiluminescent Peroxidase Substrate (120 mL)				
4-Chloro-1-naphthol			C6788	4-Chloro-1-naphthol (30 mg)	Insoluble	Blue
DAB	D6815	Diaminobenzidine (DAB) Enhanced Liquid Substrate System	D4168	SIGMA FAST 3,3'-Diaminobenzidine tablets System (1 mL)	Insoluble	Brown
	D7304	Diaminobenzidine (DAB) Liquid Substrate System	D4293	SIGMA FAST 3,3'-Diaminobenzidine tablets System (5 mL)		
	D7679	DAB Dropper System	D4418	SIGMA FAST 3,3'-Diaminobenzidine tablets System (15 mL)		
			D5905	3,3'-Diaminobenzidine tetrahydrochloride (10 mg)		
TMB	T0565	Tetramethylbenzidine Liquid Substrate System	D0426	SIGMA FAST DAB with Metal Enhancer	Insoluble	Blue

## Immunohistochemistry

Substrate Composition	Liquid Substrate Systems		Tablets		End Product	Detection/Color
	Prod. No.	Product Description	Prod. No.	Product Description		
<b>ALKALINE PHOSPHATASE</b>						
BCIP/NBT	B1911	BCIP/NBT Liquid Substrate System, True Measure	B5655	SIGMA FAST™ BCIP/NBT System (10 mL)	Insoluble	Blue/Purple
Fast Red/Naphthol AS-MX			F4648	SIGMA FAST Fast Red TR/Naphthol AS-MX Tablets (1 mL)	Insoluble	Red
			F4523	SIGMA FAST Fast Red TR/Naphthol AS-MX Tablets (10 mL)		
			F5146	Fast Red RC Tablet (10 mg)		
			N8518	Naphthol AS-TR phosphate disodium salt (4 mg)		
(TAC) Buffer/ Hexazonium Initiator/Magenta	AM0100	Alkaline Phosphatase Magenta Substrate			Insoluble	Magenta
<b>PEROXIDASE</b>						
AEC			A6926	3-Amino-9-ethylcarbazole (20 mg)	Soluble	Brown
DAB	D3939	Diaminobenzidine (DAB) Enhanced Liquid Substrate System	D4168	SIGMA FAST 3,3'-Diaminobenzidine tablets (1 mL)	Insoluble	Brown
	D7304	Diaminobenzidine (DAB) Liquid Substrate System	D4293	SIGMA FAST 3,3'-Diaminobenzidine tablets (5 mL)		
	D7679	DAB Dropper System	D4418	SIGMA FAST 3,3'-Diaminobenzidine tablets (15 mL)		
			D0426	SIGMA FAST DAB with Metal Enhancer		
			D5905	3,3'-Diaminobenzidine tetrahydrochloride (10 mg)		



# ELISA Substrates

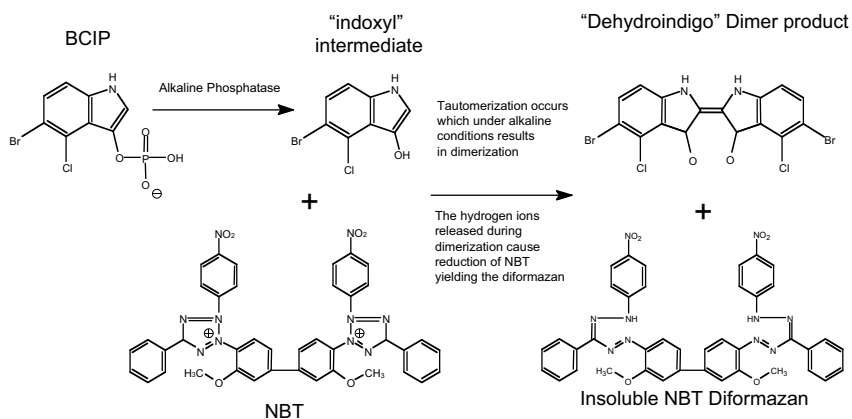
Substrate Composition	Liquid Substrate Systems		Tablets		End Product	Detection/Color
	Prod. No.	Product Description	Prod. No.	Product Description		
<b>ALKALINE PHOSPHATASE</b>						
BCIP Analog/INTX	AR0100	Alkaline Phosphatase Red Microwell Substrate			Soluble	Red
	AR0200	Alkaline Phosphatase Red Microwell Substrate				
BCIP Analog/NBT	AB0100	Alkaline Phosphatase Blue Microwell Substrate			Soluble	Blue
	AB0200	Alkaline Phosphatase Blue Microwell Substrate				
	A5852	AP Stop Reagent				
MUP	M3168	Methylumbelliferyl Phosphate (MUP)			Soluble	Fluorimetric
pNPP	A3469	Alkaline Phosphatase Yellow (pNPP)	N1891	SIGMA FAST p-Nitrophenyl phosphate tablets (5 mL)	Soluble	Yellow
			N2770	SIGMA FAST p-Nitrophenyl phosphate tablets (20 mL)		
			N9389	p-Nitrophenyl phosphate disodium salt hexahydrate tablet (5 mg)		
			N2640	p-Nitrophenyl phosphate disodium salt hexahydrate tablet (15 mg)		
			N2765	p-Nitrophenyl phosphate disodium salt hexahydrate tablet (20 mg)		
			S0942	Phosphatase Substrate (5 mg capsule)		
			P5744	Phosphatase Substrate (40 mg capsule)		
			P5994	Phosphatase Substrate (40 mg tablet)		
			P5869	Phosphatase Substrate (100 mg capsule)		
<b>PEROXIDASE</b>						
AzBTS	A3219	AzBTS Liquid Substrate System	A9941	AzBTS Tablet (10 mg)	Soluble	Green
	A1227	AzBTS Enhancer				
5-Aminosalicylic acid			A6178	5-Aminosalicylic acid (100 mg)	Soluble	Brown
Chemiluminescent	CPS2-60	Chemiluminescent Peroxidase Substrate for ELISA (60 mL)			Soluble	Chemiluminescent
	CPS2-120	Chemiluminescent Peroxidase Substrate for ELISA (120 mL)				
	CPS2-300	Chemiluminescent Peroxidase Substrate for ELISA (300 mL)				
o-Dianisidine			D9154	o-Dianisidine dihydrochloride	Soluble	Yellow-Orange
OPD			P9187	SIGMA FAST OPD System (20 mL)	Soluble	Orange
			P5412	o-Phenylenediamine (20 mg)		
			P6662	o-Phenylenediamine diHCl (1 mg)		
			P6787	o-Phenylenediamine diHCl (2 mg)		
			P8806	o-Phenylenediamine diHCl (3 mg)		
			P8787	o-Phenylenediamine diHCl (4 mg)		
			P3804	o-Phenylenediamine diHCl (5 mg) (16 mg total Weight)		
			P6912	o-Phenylenediamine diHCl (5 mg) (150 mg total weight)		
			P8287	o-Phenylenediamine diHCl (10 mg)		
			P4664	o-Phenylenediamine diHCl (15 mg)		
			P7288	o-Phenylenediamine diHCl (20 mg)		
			P8412	o-Phenylenediamine diHCl (30 mg)		
			P1063	o-Phenylenediamine diHCl (60 mg)		
TMB	T8665	Tetramethylbenzidine Liquid Substrate System	T3405	3,3',5,5'-Tetramethylbenzidine dihydrochloride (1 mg)	Soluble	Blue
	T0440	Tetramethylbenzidine Liquid Substrate System	T5525	3,3',5,5'-Tetramethylbenzidine (1 mg)		
	T4444	Tetramethylbenzidine Liquid Substrate, Supersensitive				
	T4319	Tetramethylbenzidine Liquid Substrate, Slow Kinetic Form				
	T5569	Tetramethylbenzidine Liquid Substrate, Super Slow Kinetic Form				
	S5689	Stop reagent, TMB Substrate for ELISA, 650 nm				
S5814	Stop reagent, TMB Substrate for ELISA, 450 nm					

# Colorimetric Alkaline Phosphatase and Peroxidase Substrate Detection Systems

## Substrates for Alkaline Phosphatase

### Nitroblue Tetrazolium (NBT)

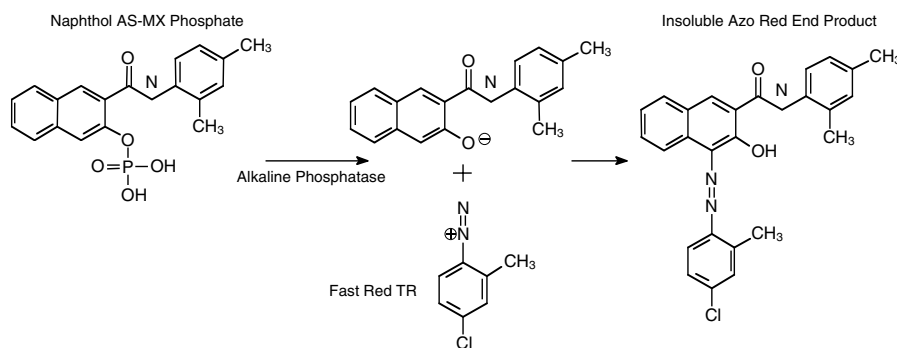
is used with the alkaline phosphatase substrate 5-Bromo-4-Chloro-3-Indolyl Phosphate (BCIP) in western blotting and immunohistological staining procedures. These substrate systems produce an insoluble NBT diformazan end product that is blue to purple in color and can be observed visually. Immunohistochemical staining with BCIP/NBT requires organic mounting media and can be counterstained with Nuclear Fast Red or Light Green. BCIP/NBT is generally a more sensitive method than Fast Red.



Sigma also offers proprietary formulations of BCIP analogs that utilize NBT and INTX to produce soluble end products specifically for ELISA applications.

### Fast Red TR/Naphthol AS-MX and TR phosphate

(4-Chloro-2-methyl-benzenediazonium/3-Hydroxy-2-naphthoic acid 2,4-dimethylanilide phosphate) substrate systems have been formulated and optimized for use in immunohistology and western blotting as a precipitating substrate for the detection of alkaline phosphatase activity. Fast Red systems produce an insoluble intense red end product. Slides stained with Fast Red TR/Naphthol AS-MX must be coverslipped using aqueous mounting media as the reaction product is alcohol soluble.



**pNPP substrates** develop the yellow end-product, p-nitrophenol, when hydrolyzed by alkaline phosphatase. Since this system forms a soluble end product, it is suitable for ELISA, but not recommended for blotting or histochemistry. These products are supplied as ready-to-use buffered alkaline phosphatase substrate solutions containing p-NPP. The soluble yellow end product absorbs at 405 nm. The reaction may be stopped with the addition of 3 M sodium hydroxide solution. ELISA applications utilizing pNPP may be read in timed assays or stopped with alkaline solutions for delayed readings. The absorbance of the stopped reaction is also read at 405 nm. For ELISA applications, typically 200 microliters of substrate is added per well of the ELISA plate and the reaction is stopped with 50 microliters of 3M NaOH solution.



## Blotting

### Kits

#### ProteoQwest™ Colorimetric Western Blotting Kit, BCIP®/NBT Substrate

##### ► for Mouse Monoclonal IgG Antibodies

ProteoQwest Colorimetric Western Blotting Kit, BCIP/NBT Substrate is designed for high sensitivity colorimetric detection of as little as 0.25 ng of protein. The colorimetric reaction occurs directly on the PVDF or nitrocellulose membrane; no darkroom or film is needed. All of the components of this ProteoQwest kit have been extensively tested and optimized. This kit is designed for 25 mini-gel sized (10 × 10 cm) blots. It is possible to use this kit for as many as 45 blots if half the suggested amount of reagents is used.

##### Features and Benefits

- Short procedure time
- Reproducible results
- Minimal background

##### Components

Chemichrome™ Western Control (Sigma C2242) 1 vial  
Tris Buffered Saline, pH 8.0, with 3% nonfat Milk (Sigma T8793) 25 packets  
Tris Buffered Saline with TWEEN 20 25 packets  
Anti-Mouse IgG (whole molecule)–Alkaline Phosphatase antibody produced in goat (Sigma A3562) 1 vial  
BCIP®/NBT solution, premixed (Sigma B6404) 100 mL  
store at: 2–8°C

PQ0111-1KT

1 kit

### Liquid Systems

Specifically formulated liquid enzyme substrates which are stable and optimized for blotting applications offer maximum convenience, time savings and consistency.

#### Alkaline Phosphatase Blue Membrane Substrate Solution

Prior to reaction with alkaline phosphatase, the BCIP reagent is colorless to faint blue and the Nitro BT reagent is yellow. The two-component mixture develops a bluish-purple product with alkaline phosphatase in membrane-type assays. This substrate is not recommended for ELISA assays.

A 2 component kit sufficient for preparing 400 mL of working substrate.  
store at: 2–8°C

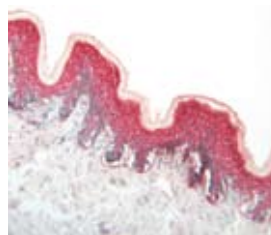
AB0300-1KT

1 kit

A 2 component kit sufficient for preparing 2 liters of working substrate.  
store at: 2–8°C

AB0400-1KT

1 kit



#### Alkaline Phosphatase Magenta® Immunohistochemical Substrate Solution

A three component buffered immunohistochemical substrate for alkaline phosphatase containing modified Gormori's Tris Azo Coupling (TAC) Buffer, hexazonium salt initiator, and Magenta. The TAC Buffer coupled with naphthol is hydrolyzed by the alkaline phosphatase. This hydrolyzed product, in the presence of hexazonized new magenta, produces a fine magenta precipitate at the site of alkaline phosphatase activity suitable for membrane-type procedures.

store at: 2–8°C

AM0100-1KT

1 kit

#### Alkaline Phosphatase Red Membrane Substrate Solution

Supplied as a 2 component buffered alkaline phosphatase substrate containing BCIP and INTX

Sufficient for preparing 400 mL of working substrate.  
store at: 2–8°C

AR0300-1KT

1 kit

Sufficient for preparing 2 liters of working substrate.  
store at: 2–8°C

AR0400-1KT

1 kit

### BCIP®/NBT- Liquid Substrate Systems for Membranes

#### BCIP®/NBT-Blue Liquid Substrate System for Membranes

store at: 2–8°C

B3804-100ML

100 mL

#### BCIP®/NBT-Purple Liquid Substrate System for Membranes

Ready-to-use.

store at: 2–8°C

B3679-100ML

100 mL

#### BCIP®/NBT solution, premixed

B6404-100ML

100 mL

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## Substrates for Alkaline Phosphatase

### Blotting, continued

#### Powder

BCIP and NBT are also available as pure powders. BCIP is provided in two salt forms: the disodium salt which is soluble in water and the *p*-toluidine form which is soluble in dimethylformamide.

#### 5-Bromo-4-chloro-3-indolyl phosphate

##### 5-Bromo-4-chloro-3-indolyl phosphate disodium salt

X-phosphate disodium salt; BCIP®  
 $C_8H_6BrClNO_4P \cdot 2Na$   $C_8H_6BrClNNa_2O_4P$  FW 370.43  
 Histochemical substrate for alkaline phosphatase.

store at: -20°C

B6149-25MG	25 mg
B6149-50MG	50 mg
B6149-100MG	100 mg
B6149-500MG	500 mg
B6149-1G	1 g
B6149-5G	5 g

##### 5-Bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt

BCIP® *p*-toluidine salt; X-phosphate *p*-toluidine salt  
 $C_8H_6BrClNO_4P \cdot C_7H_9N$  FW 433.62

store at: -20°C

B8503-25MG	25 mg
B8503-50MG	50 mg
B8503-100MG	100 mg
B8503-500MG	500 mg
B8503-1G	1 g
B8503-5G	5 g

#### 5-Bromo-4-chloro-3-indolyl phosphate ▲

##### Nitrotetrazolium Blue chloride

2,2'-bis(4-Nitrophenyl)-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenyl-ene)ditetrazolium chloride; *p*-Nitrotetrazolium blue; Nitro BT; NBT; 3,3'-(3,3'-Dimethoxy-4,4'-biphenylene)bis[2-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride]; *p*-Nitro-Blue tetrazolium chloride [298-83-9]  $C_{40}H_{30}N_{10}O_6 \cdot 2Cl$   $C_{40}H_{30}Cl_2N_{10}O_6$  FW 817.64  
 Substrate for dehydrogenases<sup>1</sup> and other oxidases.<sup>2</sup>

Lit cited: 1. *Meth. Enzymol.* **6**, 958 (1963); 2. *Anal. Biochem.* **56**, 353 (1973)

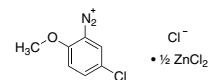
store at: 2-8°C

N6876-10X10MG	10 × 10 mg
N6876-50MG	50 mg
N6876-100MG	100 mg
N6876-250MG	250 mg
N6876-500MG	500 mg
N6876-1G	1 g
N6876-5G	5 g

#### Tablet Systems

##### Fast Red RC

[4274-03-7]



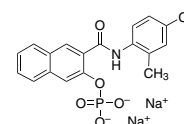
##### ▶ tablet, 10 mg substrate per tablet

Fast Red RC is used in conjunction with the alkaline phosphatase substrate Naphthol AS-TR Phosphate (Product No. N8518) in immunoblotting and immunohistological staining procedures. This substrate system provides an insoluble end product that is red in color and can be observed visually. Fast Red RC Tablets may also be used with other naphthol phosphate derivatives and in other applications.

F5146-50TAB	50 tablets
F5146-100TAB	100 tablets

##### Naphthol AS-TR phosphate disodium salt

[4264-93-1]  $C_{18}H_{13}ClNNa_2O_5P$  FW 435.71



##### ▶ tablet, 4 mg substrate per tablet

Naphthol AS-TR Phosphate is a substrate for use with alkaline phosphatase in immunoblotting and immunohistological staining procedures. When Naphthol AS-TR Phosphate is used in conjunction with Fast Red RC Tablets (Sigma Product No. F 5146) an insoluble end product is produced that is red in color and can be observed visually. Naphthol AS-TR Phosphate Tablets may also be used with other coupling salts in other applications.

store at: -20°C

N8518-50TAB	50 tablets
N8518-100TAB	100 tablets

##### SIGMAFAST™ Fast Red TR/Naphthol AS-MX Tablets

Developed for use as a precipitating substrate for the detection of alkaline phosphatase activity in immunochemistry. Primary uses are in immunohistology, immunoblotting, or dot blotting. SIGMAFAST™ Fast Red TR/Naphthol AS-MX tablets contain 0.6 mM levamisole to block endogenous alkaline phosphatase activity. SIGMAFAST™ Fast Red TR/Naphthol AS-MX Tablets require no additional steps or buffers to prepare an active substrate solution.

##### ▶ tablet, To prepare 1 mL

Each tablet dissolved in 1 mL deionized water yields a ready-to-use buffered solution.

store at: -20°C

F4648-5SET	5 sets
F4648-50SET	50 sets

##### ▶ tablet, To prepare 10 mL

Each tablet set dissolved in 10 mL of deionized water yields a ready-to-use buffered solution.

store at: -20°C

F4523-5SET	5 sets
F4523-50SET	50 sets





**Blotting, continued****5-Bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt**

BCIP® *p*-toluidine salt; X-phosphate *p*-toluidine salt  
 $C_8H_8BrClNO_4P \cdot C_7H_7N$  FW 433.62

**▶ tablet**

Substrate of choice for use with alkaline phosphatase in immunoblotting and, less commonly, in immunohistological staining procedures. High assay sensitivity is achieved via amplification when BCIP is used in conjunction with Nitro Blue Tetrazolium (NBT) Tablets (Catalog No. N5514). This substrate produces an insoluble end product that is blue-purple in color and can be observed visually.

Contains 25 mg substrate per tablet.

store at: -20°C

B0274-10TAB	10 tablets
B0274-25TAB	25 tablets

**Nitro Blue Tetrazolium**

Nitrotetrazolium Blue Chloride  
 [298-83-9]

**▶ tablet**

Nitro Blue Tetrazolium is used in conjunction with the alkaline phosphatase substrate 5-Bromo-4-Chloro-3-Indolyl Phosphate (BCIP) Tablets (Sigma Product No. B0274) in immunoblotting and immunohistological staining procedures. This substrate system produces an insoluble end product that is blue-purple in color and can be observed visually.

Contains 10 mg substrate per tablet.

store at: 2-8°C

N5514-10TAB	10 tablets
N5514-25TAB	25 tablets

**ELISA****Liquid Systems****Alkaline Phosphatase Blue Microwell Substrate**

Supplied as a 2 component buffered alkaline phosphatase substrate containing Bromo-Chloro-Indolylphosphate (BCIP) and Nitro Blue Tetrazolium (NBT/Thiazolyl Blue/Nitro BT)

A 2 component kit sufficient for preparing 400 mL of working substrate.

store at: 2-8°C

AB0100-1KT	1 kit
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A 2 component kit sufficient for preparing 2 liters of working substrate.

store at: 2-8°C

AB0200-1KT	1 kit
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**Alkaline Phosphatase Red Microwell Substrate**

Prior to reaction with alkaline phosphatase, the BCIP reagent is a colorless to faint blue solution, and the INTX reagent is a colorless to faint yellow solution. The two component mixture develops an orangish-red product when reacted with alkaline phosphatase in microwell type assays. This substrate is not recommended for membrane or immunohistochemical type assays that require a precipitation reaction product.

A 2 component kit sufficient for preparing 2 liter working substrate.

store at: 2-8°C

AR0200-1KT	1 kit
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A 2 component kit sufficient for preparing 400 mL working substrate.

store at: 2-8°C

AR0100-1KT	1 kit
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**Alkaline Phosphatase Stop Solution**

Stop solution for Alkaline Phosphatase Red and Blue microwell substrate solutions. Use 50 µl of stop solution per 200 µl of reaction mix.

Reconstitute with 100 mL of water and mix for 30-60 min.

store at: 2-8°C

A5852-100ML	100 mL
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**SIGMAFAST™ BCIP®/NBT****▶ BCIP®/NBT Alkaline Phosphatase Substrate****tablet**

SIGMA FAST™ BCIP/NBT (5-Bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium) Tablets have been developed for use in immunochemistry as a precipitating substrate for the detection of alkaline phosphatase activity. Common uses are in immunoblotting or dot blotting. It is less commonly used in immunohistochemistry. These tablets require no additional buffers or steps to prepare an active substrate solution.

Each tablet dissolved in 10 ml deionized water yields a ready-to-use buffered solution containing BCIP/NBT, pH 9.5.

store at: -20°C

B5655-5TAB	5 tablets
B5655-25TAB	25 tablets

**Alkaline Phosphatase Yellow (pNPP)  
Liquid Substrate System for ELISA**

Ready to use

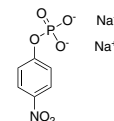
Contains *p*-nitrophenyl phosphate, a substrate for alkaline phosphatase.

store at: 2-8°C

P7998-100ML	100 mL
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***p*-Nitrophenyl Phosphate Liquid Substrate System**

4-Nitrophenyl phosphate disodium salt solution; pNPP  
 [4264-83-9]

**▶ liquid**

Substrate for alkaline phosphatase that yields a soluble yellow end product that may be read at 405 nm. The reaction may be stopped with 3N NaOH and read at 405 nm. Recommended for ELISA (microwell) procedures, not recommended for membrane applications.

Ready-to-use.

ship: wet ice store at: -20°C

N7653-100ML	100 mL
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# Substrates for Alkaline Phosphatase

## ELISA, continued

### Powder

#### ▼ 4-Nitrophenyl phosphate

##### 4-Nitrophenyl phosphate bis(cyclohexylammonium) salt

[52483-84-8]  $O_2NC_6H_4OP(O)(O)_2 \cdot 2C_6H_{11}NH_3^+ \cdot C_6H_5NO_2P \cdot 2C_6H_{13}N$   
FW 417.44

store at:  $-20^\circ\text{C}$

N3129-1G	1 g
N3129-5G	5 g
N3129-25G	25 g
N3129-100G	100 g

##### 4-Nitrophenyl phosphate bis[tris(hydroxymethyl)-aminomethane] salt

[330-13-2]  $C_{14}H_{28}N_3O_{12}P$  FW 461.36

#### ► BioChemika, for the determination of phosphatase

store at:  $2-8^\circ\text{C}$

73737-5G	5 g
73737-25G	25 g

##### 4-Nitrophenyl phosphate di(tris) salt

[68189-42-4]  $C_{14}H_{28}N_3O_{12}P$  FW 461.36

store at:  $-20^\circ\text{C}$

N3254-1G	1 g
N3254-5G	5 g
N3254-25G	25 g
N3254-100G	100 g

##### 4-Nitrophenyl phosphate magnesium salt

[32348-90-6]  $C_6H_4NO_6PMg \cdot C_6H_4MgNO_6P$  FW 241.38

store at:  $-20^\circ\text{C}$

N2507-100MG	100 mg
N2507-250MG	250 mg
N2507-1G	1 g

#### 4-Nitrophenyl phosphate ▲

##### Phosphatase substrate

4-Nitrophenyl phosphate disodium salt hexahydrate; pNPP disodium salt hexahydrate

[4264-83-9]

#### ► Bulk package

store at:  $-20^\circ\text{C}$

P4744-1G	1 g
P4744-5G	5 g
P4744-10G	10 g
P4744-25G	25 g
P4744-50G	50 g

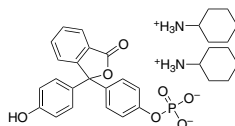
##### Phenolphthalein monophosphate bis(cyclohexylammonium) salt

[14815-59-9]  $C_{20}H_{15}O_7P \cdot 2C_6H_{13}N$   
FW 596.65

#### ► powder

store at:  $-20^\circ\text{C}$

P5758-1G	1 g
P5758-5G	5 g
P5758-10G	10 g



## Tablet Systems

pNPP tablets and capsule contents can be dissolved in either 0.1 M glycine buffer containing 1 mM  $MgCl_2$ , 1 mM  $ZnCl_2$ , pH 10.4, or 1 M diethanolamine buffer containing 0.5 mM  $MgCl_2$ , pH 9.8. pNPP is typically used at a concentration of 1 mg/ml. SIGMAFAST tablets can be dissolved in water to yield a ready-to-use buffered solution. Supplied individually foil wrapped for ease of use.

##### 4-Nitrophenyl phosphate disodium salt hexahydrate

p-Nitrophenyl phosphate disodium hexahydrate; pNPP disodium hexahydrate; Disodium 4-nitrophenyl phosphate hexahydrate; di-Sodium 4-nitrophenyl phosphate [4264-83-9]  $C_6H_4NNa_2O_6P \cdot 6H_2O$  FW 371.14

#### ► tablet

5 mg substrate per tablet

store at:  $-20^\circ\text{C}$

N9389-50TAB	50 tablets
N9389-100TAB	100 tablets

#### ► tablet

Contains 20 mg substrate per tablet

store at:  $-20^\circ\text{C}$

N2765-50TAB	50 tablets
N2765-100TAB	100 tablets

#### ► tablet

Contains 15 mg substrate per tablet

store at:  $-20^\circ\text{C}$

N2640-50TAB	50 tablets
N2640-100TAB	100 tablets

##### Phosphatase substrate

4-Nitrophenyl phosphate disodium salt hexahydrate; pNPP disodium salt hexahydrate [4264-83-9]

#### ► 5 mg tablets

store at:  $-20^\circ\text{C}$

S0942-50TAB	50 tablets
S0942-100TAB	100 tablets
S0942-200TAB	200 tablets

#### ► 40 mg tablets

Contains 40 mg substrate per tablet

store at:  $-20^\circ\text{C}$

P5994-25TAB	25 tablets
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#### ► 40 mg capsules

store at:  $-20^\circ\text{C}$

P5744-25CAP	25 caps
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#### ► 100 mg capsules

store at:  $-20^\circ\text{C}$

P5869-10CAP	10 caps
P5869-25CAP	25 caps

##### SIGMAFAST™ p-Nitrophenyl phosphate Tablets

#### ► tablet, To prepare 5 mL

Each tablet set dissolved in 5 mL deionized water yields a ready-to-use buffered solution containing pNPP.

store at:  $-20^\circ\text{C}$

N1891-5SET	5 sets
N1891-50SET	50 sets

#### ► tablet, To prepare 20 mL

Each tablet set dissolved in 20 mL deionized water yields a ready-to-use buffered solution containing pNPP.

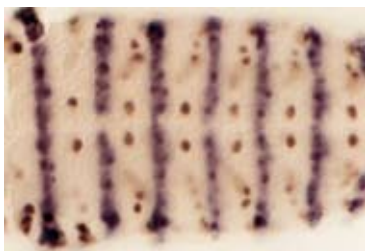
store at:  $-20^\circ\text{C}$

N2770-5SET	5 sets
N2770-50SET	50 sets



## Immunohistochemistry

## Liquid Systems



Thoracic and abdominal segments of a *Drosophila melanogaster* embryo double labeled using monoclonal antibodies to engrailed (purple) and achaete (brown) proteins. Engrailed expression marks the posterior compartment of each segment and achaete expressions labels five neuroblasts per hemisegment: three found midway between engrailed stripes and two found coincident with engrailed stripes. The cellular outlines of non-achaete positive neuroblasts are clearly visible interspersed among the achaete positive neuroblasts. Goat Anti-Mouse IgG-Alkaline Phosphatase Conjugate (Cat. No. **A7434**) and BCIP/NBT (Cat. No. **B8503** and **N6876**) and DAB tablets (Cat. No. **D5905**) were used. [From J. Skeath, Univ. of Wisc. Madison, WI]

## BCIP®/NBT Liquid Substrate System

A ready-to-use, precipitating substrate system for alkaline phosphatase. This substrate system produces an insoluble NBT diformazan end product that is blue to purple in color and can be observed visually. Immunohistochemical staining with BCIP/NBT utilizes requires organic mounting media and can be counterstained with Nuclear Fast Red or Light Green. BCIP/NBT is generally a more sensitive method than Fast Red. Not recommended for ELISA (microwell) procedures.

**B1911-100ML** 100 mL



## Alkaline Phosphatase Magenta® Immunohistochemical Substrate Solution

A three component buffered immunohistochemical substrate for alkaline phosphatase containing modified Gormori's Tris Azo Coupling (TAC) Buffer, Hexazonium Salt initiator, and Magenta. The TAC Buffer coupled with naphthol is hydrolyzed by the alkaline phosphatase. This hydrolyzed product, in the presence of hexazonized new magenta, produces a fine magenta precipitate at the site of alkaline phosphatase activity suitable for membrane-type procedures.

store at: 2-8°C

**AM0100-1KT** 1 kit

## Powder

## 5-Bromo-4-chloro-3-indolyl phosphate dipotassium salt

X-phosphate dipotassium salt; BCIP®

[102185-49-9]  $C_8H_4BrClNO_4PK_2$   $C_8H_4BrClK_2NO_4P$  FW 402.65

Histochemical substrate for alkaline phosphatase.

store at: -20°C

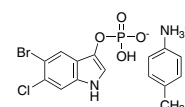
<b>B6274-25MG</b>	25 mg
<b>B6274-50MG</b>	50 mg
<b>B6274-100MG</b>	100 mg
<b>B6274-500MG</b>	500 mg
<b>B6274-1G</b>	1 g

## 5-Bromo-6-chloro-3-indolyl phosphate p-toluidine salt

Magenta® phosphate

[6769-80-8]  $C_8H_6BrClNO_4P \cdot C_7H_9N$

FW 433.62

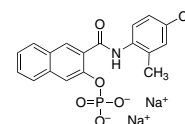


store at: -20°C

<b>B5667-25MG</b>	25 mg
<b>B5667-100MG</b>	100 mg

## Naphthol AS-TR phosphate disodium salt

[4264-93-1]  $C_{18}H_{13}ClINNa_2O_5P$  FW 435.71



## ► Bulk package

store at: -20°C

<b>N6125-100MG</b>	100 mg
<b>N6125-1G</b>	1 g
<b>N6125-5G</b>	5 g

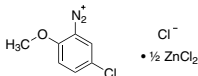
# Substrates for Alkaline Phosphatase

## Immunohistochemistry, continued

### Tablet Systems

#### Fast Red RC

[4274-03-7]



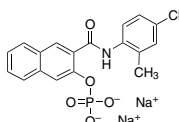
► **tablet, 10 mg substrate per tablet**

Fast Red RC is used in conjunction with the alkaline phosphatase substrate Naphthol AS-TR Phosphate (Product No. N8518) in immunoblotting and immunohistological staining procedures. This substrate system provides an insoluble end product that is red in color and can be observed visually. Fast Red RC Tablets may also be used with other naphthol phosphate derivatives and in other applications.

F5146-50TAB	50 tablets
F5146-100TAB	100 tablets

#### Naphthol AS-TR phosphate disodium salt

[4264-93-1] C<sub>18</sub>H<sub>13</sub>ClINNa<sub>2</sub>O<sub>5</sub>P FW 435.71



► **tablet, 4 mg substrate per tablet**

Naphthol AS-TR Phosphate is a substrate for use with alkaline phosphatase in immunoblotting and immunohistological staining procedures. When Naphthol AS-TR Phosphate is used in conjunction with Fast Red RC Tablets (Sigma Product No. F 5146) an insoluble end product is produced that is red in color and can be observed visually. Naphthol AS-TR Phosphate Tablets may also be used with other coupling salts in other applications.

store at: -20°C

N8518-50TAB	50 tablets
N8518-100TAB	100 tablets

#### SIGMAFAST™ BCIP®/NBT

► **BCIP®/NBT Alkaline Phosphatase Substrate**

**tablet**

SIGMA FAST™ BCIP/NBT (5-Bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium) Tablets have been developed for use in immunochemistry as a precipitating substrate for the detection of alkaline phosphatase activity. Common uses are in immunoblotting or dot blotting. It is less commonly used in immunohistochemistry. These tablets require no additional buffers or steps to prepare an active substrate solution.

Each tablet dissolved in 10 ml deionized water yields a ready-to-use buffered solution containing BCIP/NBT, pH 9.5.

store at: -20°C

B5655-5TAB	5 tablets
B5655-25TAB	25 tablets

#### SIGMAFAST™ Fast Red TR/Naphthol AS-MX Tablets

Developed for use as a precipitating substrate for the detection of alkaline phosphatase activity in immunochemistry. Primary uses are in immunohistology, immunoblotting, or dot blotting. SIGMAFAST™ Fast Red TR/Naphthol AS-MX tablets contain 0.6 mM levamisole to block endogenous alkaline phosphatase activity. SIGMAFAST™ Fast Red TR/Naphthol AS-MX Tablets require no additional steps or buffers to prepare an active substrate solution.

► **tablet, To prepare 1 mL**

Each tablet dissolved in 1 mL deionized water yields a ready-to-use buffered solution.

store at: -20°C

F4648-5SET	5 sets
F4648-50SET	50 sets

► **tablet, To prepare 10 mL**

Each tablet set dissolved in 10 mL of deionized water yields a ready-to-use buffered solution.

store at: -20°C

F4523-5SET	5 sets
F4523-50SET	50 sets



# Substrates for Peroxidase

## Blotting

### Kit

#### ProteoQwest™ Colorimetric Western Blotting Kit, TMB Substrate

##### ► (for Rabbit Antibodies)

The ProteoQwest Colorimetric Western Blotting Kit, TMB Substrate, is designed for high sensitivity, low background colorimetric detection of as little as 0.25 ng of protein. The colorimetric reaction occurs directly on the PVDF or nitrocellulose membrane; no darkroom or film is needed. All of the components of this ProteoQwest kit have been extensively tested and optimized. This kit is designed for 25 mini-gel sized blots. It is possible to use this kit for as many as 45 blots if half the suggested amount of reagents is used.

##### Features and Benefits

Benefits include:

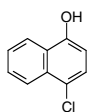
- Short procedure time
- Reproducible results
- Minimal background

##### Components

Chemichrome™ Ultimate (Sigma C2117) 200 µL  
Tris Buffered Saline with TWEEN® 20 25 packets  
Tris Buffered Saline, pH 8.0, with 3% nonfat Milk (Sigma T8793) 25 packets  
Anti-Rabbit IgG (whole molecule)-Peroxidase, ProteoQwest™ antibody produced in goat 250 µL  
TMB Liquid Substrate (Sigma B0545) 100 mL  
store at: 2-8°C

PQ0121-1KT 1 kit

### Liquid Systems



4-Chloro-1-Naphthol

#### 4-Chloro-1-naphthol solution

Substrate for visualizing peroxidase conjugates in Western blotting.

store at: 2-8°C

C8302-100ML 100 mL

#### 3,3'-Diaminobenzidine (DAB) Liquid Substrate System tetrahydrochloride

Produces a brown precipitate with peroxidase for use in immunohistological and immunoblotting staining procedures. Not recommended for ELISA (microwell) procedures.

Each DAB Liquid Substrate System will provide 250 mL of DAB substrate solution.

##### Components

10X DAB Liquid Chromogen 25 mL  
ready-to-use buffer/peroxide solution 250 mL  
ship: wet ice store at: 2-8°C

D7304-1SET 1 set

#### 3,3'-Diaminobenzidine (DAB) Enhanced Liquid Substrate System tetrahydrochloride

The DAB (3,3'-Diaminobenzidine tetrahydrochloride) Two-component Enhanced Liquid Substrate System is a precipitating substrate system for peroxidase. DAB Two-component Liquid Substrate System produces a brown precipitate for use in immunohistological and immunoblotting staining procedures.

##### ► for Membrane Applications

Each DAB Two-component Liquid Substrate System will provide 500 mL of the DAB substrate solution.

Set consists of 4 x 100 ml Solution A and 15 ml Solution B.

##### Components

3, 3'-Diaminobenzidine enhanced liquid substrate system 15 mL  
3, 3'-Diaminobenzidine enhanced liquid substrate system 4 x 100 mL  
ship: wet ice store at: 2-8°C

D6815-1SET 1 set



#### 3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate System for Membranes

TMB is a substrate for horseradish peroxidase. Develops a permanent, insoluble, dark blue reaction product. Recommended for membrane applications. Not recommended for ELISA (microwell) procedures.

Ready-to-use.

store at: 2-8°C

T0565-100ML 100 mL

### Powder

#### 3-Amino-9-ethylcarbazole

AEC; 9-Ethylcarbazol-3-amine

C<sub>14</sub>H<sub>14</sub>N<sub>2</sub> FW 210.27

Actual content given on label

Substrate for peroxidase.

store at: 2-8°C

A5754-10G 10 g

A5754-50G 50 g

A5754-100G 100 g

## Substrates for Peroxidase

### Blotting, continued

#### Tablet Systems

##### 3-Amino-9-ethylcarbazole

AEC; 9-Ethylcarbazol-3-amine  
 $C_{14}H_{14}N_2$  FW 210.27

► **tablet**

Contains 20 mg substrate per tablet.

A6926-5TAB	5 tablets
A6926-50TAB	50 tablets
A6926-100TAB	100 tablets

##### 4-Chloro-1-naphthol

4C1N  
 $ClC_{10}H_6OH$   $C_{10}H_7ClO$  FW 178.61

► **tablet**

4-Chloro-1-Naphthol is a peroxidase substrate suitable for use in immunoblotting procedures. This substrate produces an insoluble end product that is blue in color and can be observed visually.

Contains 30 mg substrate per tablet.

Dissolve 1 tablet in 10 ml of methanol. Add 2 ml of methanol stock solution to 10 ml of triethanolamine buffered saline, pH 7.5. Add 5  $\mu$ l of fresh 30% hydrogen peroxide (Sigma Product No. H-1009) immediately prior to use.

store at:  $-20^{\circ}C$

C6788-5TAB	5 tablets
C6788-50TAB	50 tablets
C6788-100TAB	100 tablets





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Anti-BID Cat No. HPA000722 on A-431 cells shown in green, nucleus in blue, microtubules in red and endoplasmic reticulum (ER) in yellow.

Just one of the over 500 IF, IHC and WB images available

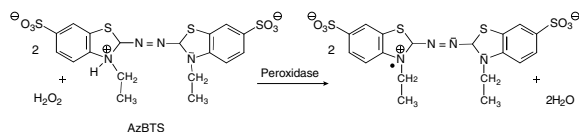
[sigma-aldrich.com](http://sigma-aldrich.com)
**SIGMA-ALDRICH®**



## ELISA

## Liquid Systems

## 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)



## ► Liquid Substrate System

Ready-to-use.

store at: 2-8°C

A3219-100ML 100 mL

## ABTS Enhancer

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Enhancer

For microwell applications, add 1 mL of ABTS Enhancer to 10 mL of ABTS. Mix gently for about one minute and use 100  $\mu$ l per well. Following the reaction, a yellow product forms that may be read at a maximum wavelength between 400 nm to 410 nm. Absorbance values of the reaction should be monitored and read before an absorbance value of 2.0 is attained.

Supplied as an 11X concentrate

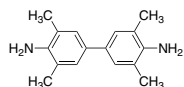
store at: 2-8°C

A1227-10ML 10 mL

## TMB Liquid Systems for ELISA



## 3,3',5,5'-Tetramethylbenzidine Liquid Substrate, Supersensitive, for ELISA



3,3',5,5'-Tetramethylbenzidine (TMB) is a peroxidase substrate suitable for use in ELISA procedures. The substrate produces a soluble end product that is pale blue in color and can be read spectrophotometrically at 370 or 655 nm. The TMB reaction may be stopped with 2 M  $H_2SO_4$  (resulting in a yellow color), and read at 450 nm.

Contains TMB in a mildly acidic buffer. The substrate is supplied as a one component ready to use solution. Rate kinetics are approx. 40% faster than traditional TMB formulations. Not recommended for membrane or immunohistochemical applications that require a precipitating reaction product.

store at: 2-8°C

T4444-4X100ML 4 x 100 mL

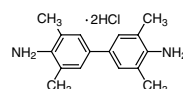
## 3,3',5,5'-Tetramethylbenzidine Liquid Substrate, Super Slow, for ELISA

Contains TMB in a mildly acidic buffer. The substrate is supplied as a one component ready to use solution. Rate kinetics are approx. 60% slower than traditional TMB formulations. Not recommended for membrane or immunohistochemical applications that require a precipitating reaction product.

store at: 2-8°C

T5569-4X100ML 4 x 100 mL

## 3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate System



TMB is a substrate for horseradish peroxidase. Forms a soluble blue end product that may be read at 370 nm or 655 nm. The TMB reaction may be stopped with 0.5 M  $H_2SO_4$ , resulting in a yellow color, and read at 450 nm. Recommended for ELISA (microwell) procedures, not recommended for membrane applications.

Ready-to-use.

store at: 2-8°C

T8665-100ML 100 mL

T8665-1L 1 L

## 3,3',5,5'-Tetramethylbenzidine Liquid Substrate, Slow Kinetic Form, for ELISA

Contains TMB in a mildly acidic buffer. The substrate is supplied as a one component ready to use solution. Rate kinetics are approx. 25% slower than traditional TMB formulations. Not recommended for membrane or immunohistochemical applications that require a precipitating reaction product.

store at: 2-8°C

T4319-4X100ML 4 x 100 mL

# Substrates for Peroxidase

## ELISA, continued

### Powder

#### 5-Aminosalicylic acid

Mesalamine; 5-AS; 5-Amino-2-hydroxybenzoic acid  
[89-57-6]  $\text{H}_2\text{NC}_6\text{H}_3\text{-2-(OH)CO}_2\text{H}$   $\text{C}_7\text{H}_7\text{NO}_3$  FW 153.14

5-Aminosalicylic acid is a peroxidase substrate suitable for use in ELISA procedures. This substrate produces a soluble end product that is brown in color and can be read spectrophotometrically at 450 nm. The reaction may be stopped with 3 N NaOH and read at 550 nm.

store at: 2-8°C

A3537-25G	25 g
A3537-100G	100 g

#### 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

AzBTS-( $\text{NH}_4$ )<sub>2</sub>; Diammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)

$\text{C}_{18}\text{H}_{24}\text{N}_6\text{O}_6\text{S}_4$  FW 548.68

A1888-1G	1 g
A1888-2G	2 g
A1888-5G	5 g

#### 4-Chloro-1-naphthol

4C1N

$\text{ClC}_{10}\text{H}_6\text{OH}$   $\text{C}_{10}\text{H}_7\text{ClO}$  FW 178.61

Chromogenic peroxidase substrate useful in enzyme-linked detection procedures.

store at: -20°C

C8890-2.5G	2.5 g
C8890-5G	5 g
C8890-25G	25 g
C8890-100G	100 g

#### o-Dianisidine

Fast Blue B

Probably carcinogenic.

#### o-Dianisidine

3,3'-Dimethoxybenzidine

[119-90-4]  $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_2$  FW 244.29

D9143-5G	5 g
D9143-25G	25 g

#### o-Dianisidine dihydrochloride

3,3'-Dimethoxybenzidine dihydrochloride

$\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_2 \cdot 2\text{HCl}$  FW 317.21

o-Dianisidine (3,3'-dimethoxybenzidine) is a peroxidase substrate suitable for use in ELISA procedures. This substrate produces a soluble end product that is yellow-orange in color and can be read spectrophotometrically at 405 nm. The reaction may be stopped with 5 M HCl.

#### ► Purified for use with peroxidase and peroxidase-coupled reactions.

store at: 2-8°C

D3252-5G	5 g
D3252-25G	25 g
D3252-100G	100 g

#### o-Dianisidine ▲

#### ▼ o-Phenylenediamine

#### o-Phenylenediamine

OPD; 1,2-Diaminobenzene

$\text{C}_6\text{H}_8\text{N}_2$  FW 108.14

store at: 2-8°C

P9029-50G	50 g
P9029-100G	100 g

#### o-Phenylenediamine dihydrochloride

$\text{C}_6\text{H}_4(\text{NH}_2)_2 \cdot 2\text{HCl}$   $\text{C}_6\text{H}_8\text{N}_2 \cdot 2\text{HCl}$  FW 181.06

store at: -20°C

P1526-10G	10 g
P1526-25G	25 g
P1526-50G	50 g
P1526-100G	100 g
P1526-1KG	1 kg

#### o-Phenylenediamine ▲

#### ▼ 3,3',5,5'-Tetramethylbenzidine

#### 3,3',5,5'-Tetramethylbenzidine

TMB

$[-\text{C}_6\text{H}_2(\text{CH}_3)_2\text{-4-NH}_2]_2$   $\text{C}_{16}\text{H}_{20}\text{N}_2$  FW 240.34

store at: 2-8°C

T2885-100MG	100 mg
T2885-1G	1 g
T2885-5G	5 g
T2885-100G	100 g

#### 3,3',5,5'-Tetramethylbenzidine dihydrochloride hydrate

TMB dihydrochloride

[207738-08-7]  $[-\text{C}_6\text{H}_2(\text{CH}_3)_2\text{-4-NH}_2]_2 \cdot 2\text{HCl} \cdot x\text{H}_2\text{O}$   $\text{C}_{16}\text{H}_{20}\text{N}_2 \cdot 2\text{HCl} \cdot x\text{H}_2\text{O}$   
FW 313.27 (Anh)

#### ► Bulk package

store at: 2-8°C

T8768-100MG	100 mg
T8768-1G	1 g
T8768-5G	5 g

#### 3,3',5,5'-Tetramethylbenzidine ▲





## ELISA, continued

## Tablet Systems

**2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt**

AzBTS-(NH<sub>4</sub>)<sub>2</sub>; Diammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)

C<sub>18</sub>H<sub>24</sub>N<sub>6</sub>O<sub>6</sub>S<sub>4</sub> FW 548.68

▶ **tablet, 10 mg substrate per tablet**

Dissolve 1 tablet in 100 ml of 0.05 M phosphate-citrate buffer, pH 5.0 (Tablets available as Sigma Product No. P4809). Add 25 µl of fresh 30% hydrogen peroxide

A9941-5TAB	5 tablets
A9941-50TAB	50 tablets
A9941-100TAB	100 tablets

**4-Chloro-1-naphthol**

4C1N

C<sub>10</sub>H<sub>7</sub>ClO C<sub>10</sub>H<sub>7</sub>ClO FW 178.61

▶ **tablet**

4-Chloro-1-Naphthol is a peroxidase substrate suitable for use in immunoblotting procedures. This substrate produces an insoluble end product that is blue in color and can be observed visually.

Contains 30 mg substrate per tablet.

Dissolve 1 tablet in 10 ml of methanol. Add 2 ml of methanol stock solution to 10 ml of triethanolamine buffered saline, pH 7.5. Add 5 µl of fresh 30% hydrogen peroxide (Sigma Product No. H-1009) immediately prior to use.

store at: -20°C

C6788-5TAB	5 tablets
C6788-50TAB	50 tablets
C6788-100TAB	100 tablets

**o-Dianisidine dihydrochloride**

Fast Blue B; 3,3'-Dimethoxybenzidine dihydrochloride

C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub> · 2HCl FW 317.21

o-Dianisidine (3,3'-dimethoxybenzidine) is a peroxidase substrate suitable for use in ELISA procedures. This substrate produces a soluble end product that is yellow-orange in color and can be read spectrophotometrically at 405 nm. The reaction may be stopped with 5 M HCl.

Probably carcinogenic.

▶ **tablet, 10 mg substrate per tablet**

Dissolve one tablet in 60 ml of 50 mM phosphate-citrate buffer, pH 5.0. Add 12 µl of fresh 30% hydrogen peroxide immediately before to use.

store at: 2-8°C

D9154-50TAB	50 tablets
D9154-100TAB	100 tablets

**SIGMAFAST™ OPD**▶ **OPD Peroxidase Substrate****tablet**

SIGMAFAST™ OPD (o-Phenylenediamine dihydrochloride) Tablets have been developed for use as a soluble substrate for the detection of peroxidase activity in enzyme immunoassays (EIA). OPD is the EIA substrate of choice as it exhibits high sensitivity. EIA applications utilizing OPD may be read in timed assays or stopped with dilute acid solutions for delayed readings.

**Features and Benefits**

SIGMAFAST OPD Tablets require no additional buffers or steps to prepare an active substrate solution.

One OPD tablet and one urea hydrogen peroxide/buffer tablet, dissolved in 20 ml of water, provides 20 ml of ready-to-use substrate. When the tablet is dissolved in this manner, the final concentrations will be 0.4 mg/ml OPD, 0.4 mg/ml urea hydrogen peroxide, and 0.05 M phosphate-citrate, pH 5.0. Tablets are individually packaged in foil packets.

Each tablet set dissolved in 20 mL deionized water yields a ready-to-use buffered solution containing OPD and urea hydrogen peroxide.

store at: 2-8°C

P9187-5SET	5 sets
P9187-50SET	50 sets

▼ **o-Phenylenediamine**

Dissolve one tablet in 0.05 M phosphate-citrate buffer, pH 5.0, to the desired concentration (typically an OPD concentration of 0.4 mg/ml is used). Add 40 µl of fresh 30% hydrogen peroxide per 100 ml of substrate buffer solution, immediately prior to use. Tablets are individually packaged in foil packets.

**o-Phenylenediamine**

OPD; 1,2-Diaminobenzene

C<sub>6</sub>H<sub>8</sub>N<sub>2</sub> FW 108.14

▶ **tablet, 20 mg substrate per tablet**

o-Phenylenediamine Free Base is a peroxidase substrate suitable for use in ELISA procedures. The substrate produces a soluble end product that is orange-brown in color and can be read spectrophotometrically at 450 nm. The OPD reaction may be stopped with 3 N HCl or 3 M H<sub>2</sub>SO<sub>4</sub> and read at 492 nm.

store at: 2-8°C

P5412-50TAB	50 tablets
P5412-100TAB	100 tablets

**o-Phenylenediamine dihydrochloride**

C<sub>6</sub>H<sub>4</sub>(NH<sub>2</sub>)<sub>2</sub> · 2HCl C<sub>6</sub>H<sub>8</sub>N<sub>2</sub> · 2HCl FW 181.06

▶ **tablet, 1 mg substrate per tablet**

store at: 2-8°C

P6662-50TAB	50 tablets
P6662-100TAB	100 tablets

▶ **tablet, 2 mg substrate per tablet**

store at: 2-8°C

P6787-50TAB	50 tablets
P6787-100TAB	100 tablets

▶ **tablet, 3 mg substrate per tablet**

store at: 2-8°C

P8806-50TAB	50 tablets
P8806-100TAB	100 tablets

▶ **tablet, 4 mg substrate per tablet**

store at: 2-8°C

P8787-50TAB	50 tablets
P8787-100TAB	100 tablets

▶ **tablet, 5 mg substrate per tablet**

16 mg total tablet weight

store at: 2-8°C

P3804-50TAB	50 tablets
P3804-100TAB	100 tablets

▶ **tablet, 5 mg substrate per tablet**

150 mg total tablet weight

store at: 2-8°C

P6912-50TAB	50 tablets
P6912-100TAB	100 tablets

## Substrates for Peroxidase

### ELISA, continued

#### ▶ tablet, 10 mg substrate per tablet

store at: 2-8°C

P8287-50TAB	50 tablets
P8287-100TAB	100 tablets

#### ▶ tablet, 15 mg substrate per tablet

store at: 2-8°C

P4664-50TAB	50 tablets
P4664-100TAB	100 tablets

#### ▶ tablet, 20 mg substrate per tablet

store at: 2-8°C

P7288-50TAB	50 tablets
P7288-100TAB	100 tablets

#### ▶ tablet, 30 mg substrate per tablet

store at: 2-8°C

P8412-50TAB	50 tablets
P8412-100TAB	100 tablets

#### ▶ tablet, 60 mg substrate per tablet

store at: 2-8°C

P1063-50TAB	50 tablets
P1063-100TAB	100 tablets

### **o-Phenylenediamine ▲**

## Immunohistochemistry

### ISOPAC®

#### 3,3'-Diaminobenzidine tetrahydrochloride

3,3',4,4'-Tetraaminobiphenyl tetrahydrochloride; 3,3',4,4'-Biphenyltetraamine tetrahydrochloride; DAB

$(\text{NH}_2)_2\text{C}_6\text{H}_3\text{C}_6\text{H}_3(\text{NH}_2)_2 \cdot 4\text{HCl}$   $\text{C}_{12}\text{H}_{14}\text{N}_4 \cdot 4\text{HCl}$  FW 360.11

Peroxidase substrate suitable for use in immunoblotting and immunohistological staining procedures. This substrate produces an insoluble end product that is brown in color and can be observed visually.

#### ▶ ISOPAC®

Weight approximate. Packaged in a 100 mL serum bottle with butyl rubber stopper and aluminum tear seal.

Dissolving the contents in 100 mL of solvent yields a 0.1% solution.

D9015-100MG	100 mg
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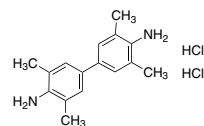
### Liquid Systems

**DAB (3,3'-Diaminobenzidine)** substrate systems are typically used for immunohistochemistry procedures as a precipitating substrate for the detection of peroxidase activity. DAB produces an intense brown stain that is easily observed visually. The addition of nickel, cobalt, or silver ions has been found to increase the intensity of DAB staining. Sigma offers several tablets and preformulated liquid forms of DAB, including systems that contain a metal enhancer. The end product is resistant to alcohol, therefore, a variety of counterstains in alcoholic solutions may be used with these DAB systems.

The DAB (3,3'-diaminobenzidine tetrahydrochloride) Liquid Substrate Systems are supplied as two reagents, the buffer and the chromogen needed to produce a fast and convenient DAB substrate solution.

### ▼ 3,3',5,5'-Tetramethylbenzidine

#### 3,3',5,5'-Tetramethylbenzidine dihydrochloride



4,4'-Diamino-3,3',5,5'-tetramethylbiphenyl dihydrochloride

[64285-73-0]  $\text{C}_{16}\text{H}_{20}\text{N}_2 \cdot 2\text{HCl}$  FW 313.27

3,3',5,5'-Tetramethylbenzidine (TMB) is a peroxidase substrate suitable for use in ELISA procedures. The substrate produces a soluble end product that is pale blue in color and can be read spectrophotometrically at 370 or 620-650 nm. The TMB reaction may be stopped with 2 M  $\text{H}_2\text{SO}_4$  (resulting in a yellow color), and read at 450 nm.

#### ▶ tablet, 1 mg substrate per tablet

store at: 2-8°C

T3405-5TAB	5 tablets
T3405-50TAB	50 tablets
T3405-100TAB	100 tablets

#### 3,3',5,5'-Tetramethylbenzidine

TMB

$[\text{-C}_6\text{H}_2(\text{CH}_3)_2\text{-4-NH}_2]_2$   $\text{C}_{16}\text{H}_{20}\text{N}_2$  FW 240.34

#### ▶ tablet, 1 mg substrate per tablet

store at: 2-8°C

T5525-50TAB	50 tablets
T5525-100TAB	100 tablets

### **3,3',5,5'-Tetramethylbenzidine ▲**

#### 3,3'-Diaminobenzidine (DAB) Enhanced Liquid Substrate System tetrahydrochloride

The DAB (3,3'-Diaminobenzidine tetrahydrochloride) Two-component Enhanced Liquid Substrate System is a precipitating substrate system for peroxidase. DAB Two-component Liquid Substrate System produces a brown precipitate for use in immunohistological and immunoblotting staining procedures.

#### ▶ for Immunohistology

Each DAB Two-component Liquid Substrate System will provide 100 mL of the DAB substrate solution.

#### Components

3, 3'-Diaminobenzidine enhanced liquid substrate system, solution B, for immuno-histochemistry 100 mL

3, 3'-Diaminobenzidine enhanced liquid substrate system, solution A, for immuno histochemical 3 mL

ship: wet ice store at: 2-8°C

D3939-1SET	1 set
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#### 3,3'-Diaminobenzidine (DAB) Liquid Substrate System tetrahydrochloride

Produces a brown precipitate with peroxidase for use in immunohistological and immunoblotting staining procedures. Not recommended for ELISA (microwell) procedures.

Each DAB Liquid Substrate System will provide 250 mL of DAB substrate solution.

#### Components

10X DAB Liquid Chromogen 25 mL

ready-to-use buffer/peroxide solution 250 mL

ship: wet ice store at: 2-8°C

D7304-1SET	1 set
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## Immunohistochemistry, continued

## Powder

## ▼ 3,3'-Diaminobenzidine

DAB; 3,3',4,4'-Biphenyltetramine  
 $(\text{NH}_2)_2\text{C}_6\text{H}_3\text{C}_6\text{H}_3(\text{NH}_2)_2$   $\text{C}_{12}\text{H}_{14}\text{N}_4$  FW 214.27

Peroxidase substrate; reagent for spectrophotometric determination of selenium.

Color: tan to brown.

store at: Room temp

D8001-1G	1 g
D8001-5G	5 g
D8001-10G	10 g
D8001-25G	25 g

## 3,3'-Diaminobenzidine tetrahydrochloride hydrate

$\text{C}_{12}\text{H}_{14}\text{N}_4 \cdot 4\text{HCl} \cdot x\text{H}_2\text{O}$  FW 360.11 (Anh)

D5637-1G	1 g
D5637-5G	5 g
D5637-10G	10 g
D5637-25G	25 g
D5637-50G	50 g

## 3,3'-Diaminobenzidine ▲

## Tablet Systems

## SIGMAFAST™ 3,3'-Diaminobenzidine tablets

Developed for use in immunohistochemistry and immunoblotting as a precipitating substrate for the localization of peroxidase activity. DAB is the immunohistochemistry substrate of choice as it produces an intense brown-black stain which is resistant to alcohol. Slides stained with DAB may be dehydrated, mounted in resinous media, and stored for future reference. SIGMAFAST™ DAB Tablets require no additional buffers or steps to prepare an active substrate solution. Tablets are individually packaged in foil packets.

## ▶ tablet, To prepare 1 mL

Each tablet set dissolved in 1 mL deionized water yields a ready-to-use buffered solution containing DAB and urea hydrogen peroxide.

store at: -20°C

D4168-5SET	5 sets
D4168-50SET	50 sets

## ▶ tablet, To prepare 5 mL

Each tablet set dissolved in 5 mL deionized water yields a ready-to-use buffered solution containing DAB and urea hydrogen peroxide.

store at: -20°C

D4293-5SET	5 sets
D4293-50SET	50 sets

## ▶ tablet, To prepare 15 mL

Each tablet set dissolved in 15 mL deionized water yields a ready-to-use buffered solution containing DAB and urea hydrogen peroxide.

store at: -20°C

D4418-5SET	5 sets
D4418-50SET	50 sets



## SIGMAFAST™ DAB with Metal Enhancer

▶ DAB Peroxidase Substrate; 3,3'-Diaminobenzidine Peroxidase Substrate

## tablet

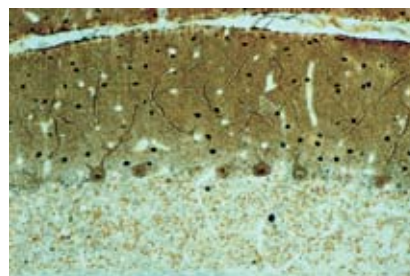
SIGMA FAST™ DAB (3,3'-Diaminobenzidine tetrahydrochloride) with Metal Enhancer Tablets have been developed for use in immunohistochemistry as a precipitating substrate for the localization of peroxidase activity. The DAB reaction is enhanced by the addition of  $\text{CoCl}_2$  and produces a more intense stain which is resistant to alcohol.

SIGMA FAST™ DAB with Metal Enhancer Tablets require no additional buffers or steps to prepare an active substrate solution for detection of peroxidase activity. Common use is in immunohistochemistry as a precipitating substrate.

Each tablet set dissolved in 5 mL deionized water yields a ready-to-use buffered solution containing DAB with a metal enhancer.

store at: -20°C

D0426-5SET	5 sets
D0426-50SET	50 sets



Taurine is located in granule cells and co-localized with GABA in Purkinje cells in 8 week-old cat cerebellum. Taurine was visualized using DAB (Cat. No. **D5905**) and GABA was stained using benzidine dihydrochloride (BDHC, Cat. No. **B3383**) as substrate. [From P. Lu, Institute for Basic Research, New York, NY]

## 3,3'-Diaminobenzidine tetrahydrochloride

3,3',4,4'-Tetraaminobiphenyl tetrahydrochloride; 3,3',4,4'-Biphenyltetraamine tetrahydrochloride; DAB

$(\text{NH}_2)_2\text{C}_6\text{H}_3\text{C}_6\text{H}_3(\text{NH}_2)_2 \cdot 4\text{HCl}$   $\text{C}_{12}\text{H}_{14}\text{N}_4 \cdot 4\text{HCl}$  FW 360.11

Peroxidase substrate suitable for use in immunoblotting and immunohistological staining procedures. This substrate produces an insoluble end product that is brown in color and can be observed visually.

## ▶ tablet, 10 mg substrate per tablet

Dissolve 1 tablet in 15 mL of Tris-buffered saline, pH 7.6. Add 12  $\mu\text{L}$  of fresh 30% hydrogen peroxide. DAB and hydrogen peroxide concentration may be adjusted to suit individual applications.

store at: -20°C

D5905-50TAB	50 tablets
D5905-100TAB	100 tablets

# Luminescent Alkaline Phosphatase and Peroxidase Substrate Detection Systems

## Substrates for Alkaline Phosphatase

### Blotting

#### Liquid Systems

CDP-*Star* is supplied as a 0.25 mM ready-to-use aqueous solution for use in a variety of membrane-based applications. CDP-*Star* functions on both neutrally-charged and positively-charged nylon, giving the reagent added application versatility.

#### CDP-*Star*<sup>®</sup> Chemiluminescent Substrate

CDP-*Star* is a sensitive, chemiluminescent substrate for alkaline phosphatase that allows for the rapid, reproducible detection of alkaline phosphatase-labeled molecules in Northern, Southern, and Western blotting applications. Detection of alkaline phosphatase-labeled molecules with CDP-*Star* is extremely sensitive as a result of low background luminescence coupled with high intensity and prolonged light output from the enzyme catalysis. Maximum light emission occurs at approximately 60 minutes and continues for up to 24 hours.

CDP-*Star*<sup>™</sup> is a trademark of Tropix, Inc. Bedford, MA, USA and covered under U.S. Patent 5,326,882 and 4,931,569.

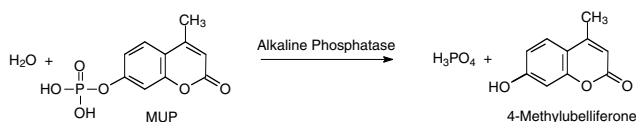
store at: 2-8°C

C0712-100ML	100 mL
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### ELISA

#### Liquid Systems

The **4-Methylumbelliferyl Phosphate (4-MUP) Liquid Substrate System** incorporates 4-Methylumbelliferyl Phosphate and buffer in a single solution, ready-to-use reagent for procedures employing alkaline phosphatase enzyme label. Fluorescence can be measured with excitation at 360 nm and emission at 440 nm. Also, the fluorescent end product may also be observed using a UV light source.



#### 4-Methylumbelliferyl phosphate (4-MUP) Liquid Substrate System

##### ► liquid

4-Methylumbelliferyl Phosphate (4-MUP) is a substrate for alkaline phosphatase which forms the soluble fluorescent substance methylumbelliferone. 4-MUP Liquid Substrate System combines 4-MUP and buffer in a ready-to-use reagent.

4-MUP fluorescence can be measured with excitation at 360 nm and emission at 440 nm. The fluorescent end product may also be observed using a UV light source.

store at: 2-8°C

M3168-100ML	100 mL
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#### Powder

The Methylumbelliferone-, Trifluoromethylumbelliferone- and the Fluorescein-fluorophore systems are available as phosphate esters in powder form.

#### Fluorescein diphosphate triammonium salt

Over 50 times more sensitive than p-nitrophenyl phosphate in detecting alkaline phosphatase in ELISA.

ship: dry ice store at: -20°C

F2678-5MG	5 mg
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#### 4-Methylumbelliferyl phosphate

4-Methylumbelliferyl-phosphoric acid

C<sub>10</sub>H<sub>9</sub>O<sub>6</sub>P FW 256.15

Fluorogenic substrate for phosphatases.

store at: -20°C

M8883-100MG	100 mg
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M8883-250MG	250 mg
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M8883-1G	1 g
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M8883-5G	5 g
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#### 4-(Trifluoromethyl)umbelliferyl phosphate disodium salt

Disodium 4-(trifluoromethyl)umbelliferyl phosphate

C<sub>10</sub>H<sub>4</sub>F<sub>3</sub>Na<sub>2</sub>O<sub>6</sub>P FW 354.08

##### ► BioChemika, for fluorescence

suitable as fluorogenic substrate for alkaline phosphatase

store at: -20°C

91882	
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### Immunohistochemistry

#### Powder

#### 4-Methylumbelliferyl phosphate disodium salt

4-MUP; Disodium 4-methylumbelliferyl phosphate

C<sub>10</sub>H<sub>7</sub>Na<sub>2</sub>O<sub>6</sub>P FW 300.11

Fluorogenic substrate for phosphatases.

store at: -20°C

M8168-250MG	250 mg
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M8168-1G	1 g
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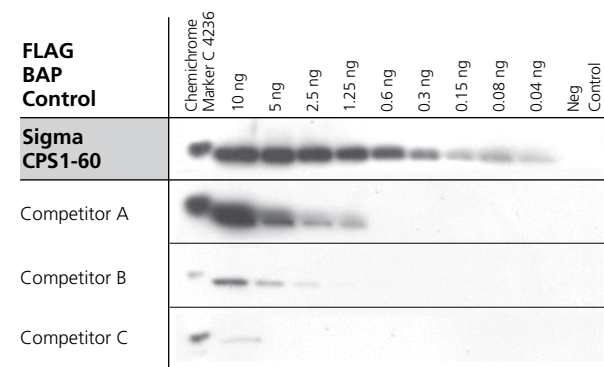
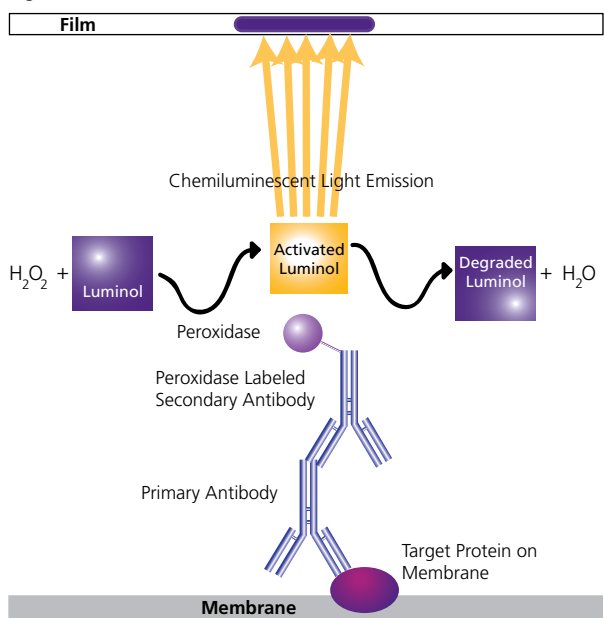


# Substrates for Peroxidase

## Blotting

Sigma's **CPS Chemiluminescent Peroxidase Substrate Systems** can be used for the highly sensitive detection of peroxidase labeled material in a variety of Western blotting and ELISA applications. These substrate systems are based on an enhanced luminol product with stabilized peroxide buffer solutions that provide maximum sensitivity with minimal background interference and superior linearity.

### Sigma CPS-1 Chemiluminescent Peroxidase Substrate



### Sigma CPS1 Versus the Competition

Chemiluminescent detection of FLAG-tagged BAP control protein in an *E. coli* lysate using Sigma CPS1 and the top three competitor products.



### Liquid Systems

CPS1 formulations provide optimal sensitivity and linearity with minimal background. Use 0.043 to 0.125 ml per cm<sup>2</sup> of membrane.

#### Chemiluminescent Peroxidase Substrate-1

CPS1 Two component liquid peroxidase substrate system for Western blot detection. Picomole detection with superior signal-to-noise ratio. Component ratios can be adjusted for optimal signal duration, sensitivity and background.

store at: 2-8°C

1 kit sufficient for 60 mL substrate

**CPS160-1KT** 1 kit

1 kit sufficient for 120 mL substrate

**CPS1120-1KT** 1 kit

1 kit sufficient for 300 mL substrate

**CPS1300-1KT** 1 kit

CPS3 Formulations are economically priced and outperform the most commonly used competitors' products. This two-component system provides low nanogram detection with superior signal-to-noise ratio. In addition, the components are stable after mixing, adding to flexibility of usage.

#### Chemiluminescent Peroxidase Substrate-3

CPS3

CPS3 formulations are economically priced and outperform the most commonly used competitors' products. This two component system provides low nanogram detection with superior signal-to-noise ratio. In addition, the components are stable after mixing, adding to the flexibility of usage.

store at: 2-8°C

1 kit sufficient for 50 mL substrate

**CPS350-1KT** 1 kit

1 kit sufficient for 100 mL substrate

**CPS3100-1KT** 1 kit

1 kit sufficient for 500 mL substrate

**CPS3500-1KT** 1 kit

## Substrates for Peroxidase

## ELISA

## Liquid Systems

## Chemiluminescent Peroxidase Substrate for ELISA

CPS2

Two component liquid substrate system with superior sensitivity, signal-to-noise, and linearity.

## Components

Chemiluminescent Reagent 20 ml  
Chemiluminescent Reaction Buffer 40 ml  
store at: 2-8°C

CPS260-1KT 1 kit

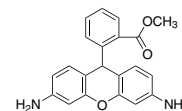
## Components

Chemiluminescent Reagent 40 ml  
Chemiluminescent Reaction Buffer 80 ml  
store at: 2-8°C

CPS2120-1KT 1 kit

## Powder

## Dihydrorhodamine 123

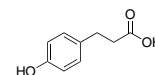
 $C_{21}H_{18}N_2O_3$  FW 346.38  
Peroxidase substrate

store at: -20°C

D1054-2MG 2 mg

D1054-10MG 10 mg

## 3-(4-Hydroxyphenyl)propionic acid

Phloretic acid  
 $C_9H_{10}O_3$  FW 166.17

## ▶ purum, for fluorescence

56190-10G 10 g

56190-50G 50 g

## Immunohistochemistry

## Powder

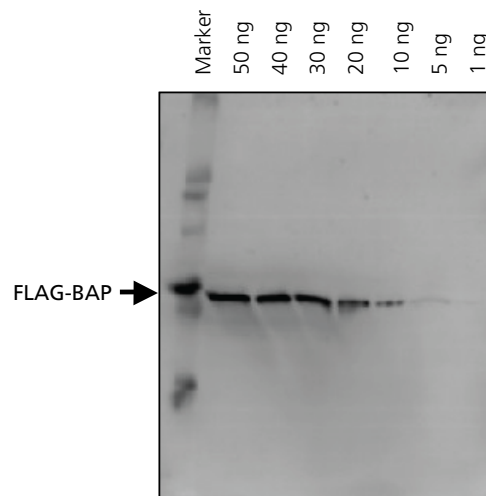
Ampliflu Red is a reliable new chemifluorescent substrate for the specific and sensitive detection of peroxidase labeled antibodies on Western blots. It is a convenient alternative to conventional colorimetric or chemiluminescent immunoblotting protocols and has the advantage of generating a high signal-to-noise ratio, without the need for long signal accumulation times. Depending on the available instrumentation, Ampliflu Red can be measured on a laser scanner or similar instruments capable of fluorescent measurements of membranes.

## Ampliflu Red

 $C_{14}H_{11}NO_4$  FW 257.24

## ▶ BioChemika, for fluorescence

90101-5MG-F 5 mg



Detection of FLAG-BAP protein (50–1 ng) by immunoblotting using Ampliflu Red (1880  $\mu$ l PBS/20  $\mu$ l Ampliflu Red from 10 mM stock/100  $\mu$ l  $H_2O_2$  from 20 mM stock). Imaging was performed on a FLA-3000 Fuji laser scanner at a fixed excitation wavelength of 532 nm with a 580 nm emission filter.



# Other Substrate Detection Systems

## Enzyme Detection by Colorimetric Methods

### Substrates for N-Acetyl- $\alpha$ -D-Galactosaminidase

Name	Cat. No.
2-Nitrophenyl N-acetyl- $\alpha$ -D-galactosaminide	N0257-5MG N0257-10MG N0257-50MG
4-Nitrophenyl N-acetyl- $\alpha$ -D-galactosaminide	N4264-5MG N4264-10MG N4264-25MG N4264-50MG

### Substrates for N-Acetyl- $\beta$ -D-Galactosaminidase

Name	Cat. No.
2-Nitrophenyl-N-acetyl- $\beta$ -D-galactosaminide	N3273-25MG
4-Nitrophenyl N-acetyl- $\beta$ -D-galactosaminide	N9003-250MG N9003-1G

### Substrates for N-Acetyl- $\alpha$ -D-Glucosaminidase

Name	Cat. No.
4-Nitrophenyl N-acetyl- $\alpha$ -D-glucosaminide	N8759-10MG N8759-25MG N8759-100MG

### Substrates for N-Acetyl- $\beta$ -D-Glucosaminidase

Name	Cat. No.
4-Nitrophenyl N-acetyl- $\beta$ -D-glucosaminide	N9376-100MG N9376-250MG N9376-500MG N9376-1G

### Substrates for Amidase

Name	Cat. No.
N $\alpha$ -Benzoyl-DL-arginine 4-nitroanilide hydrochloride	B4875-100MG B4875-500MG B4875-1G B4875-5G B4875-10G

### Substrates for Aminopeptidase A

Name	Cat. No.
L-Glutamic acid 1-(4-nitroanilide)	49622-250MG 49622-1G

### Substrates for $\alpha$ -Amylase

Name	Cat. No.
Amylopectin Azure	A4640-1G A4640-5G A4640-25G A4640-50G
Amylose-Remazol Brilliant Blue R	A3508-1G A3508-5G
4-Nitrophenyl $\alpha$ -D-maltopentaoside	N1519-25MG
4-Nitrophenyl $\alpha$ -D-maltoside	N5885-100MG
4-Nitrophenyl $\beta$ -D-maltoside	N1884-50MG N1884-250MG
Starch Azure	S7629-1G S7629-5G S7629-25G
Starch Azure	S7776-1G S7776-5G

### Substrates for $\alpha$ -L-Arabinosidase

Name	Cat. No.
4-Nitrophenyl $\alpha$ -L-arabinofuranoside	N3641-10MG N3641-25MG N3641-100MG
4-Nitrophenyl $\alpha$ -L-arabinopyranoside	N3512-1G
4-Nitrophenyl $\beta$ -L-arabinopyranoside	N0520-25MG N0520-100MG

### Substrates for Cellobiohydrolase

Name	Cat. No.
Cellulose Azure	C1052-1G C1052-5G
2-Nitrophenyl $\beta$ -D-cellobioside	N4764-100MG N4764-500MG
4-Nitrophenyl $\beta$ -D-cellobioside	N5759-100MG N5759-500MG

### Substrates for Cellulase

Name	Cat. No.
Cellulose Azure	C1052-1G C1052-5G
2-Nitrophenyl $\beta$ -D-cellobioside	N4764-100MG N4764-500MG
4-Nitrophenyl $\beta$ -D-cellobioside	N5759-100MG N5759-500MG

### Substrates for Chitinase

Name	Cat. No.
Chitin azure	C3020-100MG C3020-1G
4-Nitrophenyl N,N'-diacetyl- $\beta$ -D-chitobioside	N6133-5MG N6133-10MG
4-Nitrophenyl $\beta$ -D-N,N',N''-triacetylchitotriose	N8638-1MG N8638-5MG

### Substrates for Cholinesterase

Name	Cat. No.
2,6-Dichlorophenolindophenyl acetate	36190-10MG-F

### Substrates for Chymotrypsin

Name	Cat. No.
Ala-Ala-Phe p-nitroanilide	A9148-25MG

### Substrates for Collagenase

Name	Cat. No.
Collagenase Chromophore-Substrate (for quantitative Collagenase-Determination)	27667-10MG 27667-100MG
Collagenase Chromophore Substrate Test Substance (for quantitative Collagenase-Determination)	27668-1G
Collagenase-Substrate (for quantitative Collagenase-Determination)	27673-100MG
Collagenase-Substrate Kit (for quantitative Collagenase-Determination)	27670
Collagenase-Substrate Test Substance (for quantitative Collagenase-Determination)	27674-1G-F

### Substrates for Elastase

Name	Cat. No.
Elastin-orcein	E1500-5G
Elastin-Congo red	E0502-5G
N-Methoxysuccinyl-Ala-Ala-Pro-Val p-nitroanilide	M4765-10MG M4765-50MG M4765-250MG M4765-1G
N-Succinyl-Ala-Ala-Ala-p-nitroanilide	S4760-5MG S4760-25MG S4760-100MG S4760-500MG
N-Succinyl-Ala-Ala-Pro-Leu p-nitroanilide	S8511-10MG S8511-100MG

### Substrates for Esterase

Name	Cat. No.
5-Bromo-4-chloro-3-indolyl butyrate	B9151-5MG
5-Bromo-6-chloro-3-indolyl caprylate	53451-25MG-F 53451-100MG-F
4-Nitrophenyl acetate	N8130-5G N8130-10G N8130-25G N8130-100G

# Enzyme Detection by Colorimetric Methods

## Substrates for Exoglucanase

Name	Cat. No.
4-Nitrophenyl $\beta$ -D-cellobioside	N5759-100MG N5759-500MG

## Substrates for Factor Xa

Name	Cat. No.
N-Benzoyl-Ile-Glu-Gly-Arg p-nitroanilide acetate salt	B7020-5MG B7020-10MG
Factor Xa chromogenic substrate	F3301-25MG

## Substrates for $\alpha$ -L-Fucosidase

Name	Cat. No.
4-Nitrophenyl $\alpha$ -L-fucopyranoside	N3628-25MG N3628-100MG N3628-250MG N3628-1G

## Substrates for $\beta$ -D-Fucosidase

Name	Cat. No.
2-Nitrophenyl $\beta$ -D-fucopyranoside	N3253-25MG N3253-100MG
4-Nitrophenyl $\beta$ -D-fucopyranoside	N3378-25MG N3378-100MG N3378-250MG N3378-1G

## Substrates for Fucosyltransferase

Name	Cat. No.
4-Nitrophenyl 2-acetamido-2-deoxy-3-O- $\beta$ -D-galactopyranosyl- $\beta$ -D-glucopyranoside	N5513-1MG N5513-5MG

## Substrates for $\alpha$ -Galactosidase

Name	Cat. No.
2-Nitrophenyl $\alpha$ -D-galactopyranoside	N8888-50MG
3-Nitrophenyl $\alpha$ -D-galactopyranoside	N6129-250MG
4-Nitrophenyl $\alpha$ -D-galactopyranoside	N0877-100MG N0877-250MG N0877-500MG N0877-1G N0877-5G

## Substrates for $\beta$ -Galactosidase

Name	Cat. No.
5-Bromo-4-chloro-3-indolyl $\beta$ -D-galactopyranoside	B9146-10MG B9146-100MG B9146-500MG
5-Bromo-4-chloro-3-indolyl $\beta$ -D-galactopyranoside	B6024-10TAB B6024-50TAB
5-Bromo-3-indolyl $\beta$ -D-galactopyranoside	B4387-25MG B4387-100MG
Chlorophenol Red- $\beta$ -D-galactopyranoside	59767-25MG-F 59767-100MG-F
1-Methyl-3-indolyl- $\beta$ -D-galactopyranoside	67610-25MG 67610-100MG
4-Nitrophenyl $\beta$ -D-galactopyranoside	N1252-250MG N1252-500MG N1252-1G N1252-5G N1252-10G
4-Nitrophenyl $\beta$ -D-lactopyranoside	N1752-100MG N1752-500MG

## Substrates for $\beta$ -Galacturonidase

Name	Cat. No.
4-Nitrophenyl $\beta$ -D-galacturonide	N8755-10MG N8755-25MG

## Substrates for $\alpha$ -Glucosidase

Name	Cat. No.
4-Nitrophenyl $\alpha$ -D-glucopyranoside	N1377-1G N1377-5G N1377-25G

## Substrates for $\beta$ -Glucosidase

Name	Cat. No.
6-Bromo-2-naphthyl $\beta$ -D-glucopyranoside	B7877-1G B7877-5G
2-Chloro-4-nitrophenyl- $\beta$ -D-maltotriose	25591-100MG
2-Nitrophenyl $\beta$ -D-glucopyranoside	N8016-250MG N8016-1G N8016-5G
4-Nitrophenyl $\beta$ -D-glucopyranoside	N7006-500MG N7006-1G N7006-5G N7006-25G

## Substrates for $\beta$ -Glucuronidase

Name	Cat. No.
5-Bromo-4-chloro-3-indolyl $\beta$ -D-glucuronide sodium salt	B5285-10MG B5285-25MG B5285-100MG
5-Bromo-6-chloro-3-indolyl $\beta$ -D-glucuronide cyclohexylammonium salt	B4532-10MG
4-Nitrophenyl $\beta$ -D-glucuronide	73677-250MG 73677-1G

## Substrates for $\gamma$ -Glutamyltransferase

Name	Cat. No.
L-Glutamic acid $\gamma$ -(4-nitroanilide)	G1135-1G G1135-5G G1135-10G G1135-25G

## Substrates for O-Glycosidase

Name	Cat. No.
p-Nitrophenyl galacto-N-bioside	N3016-1MG N3016-5MG

## Substrates for HIV Protease

Name	Cat. No.
Anthranilyl-HIV Protease Substrate VI	H6160-1MG
HIV Protease Substrate 1	H6660-1MG

## Substrates for Inositolmonophosphatase

Name	Cat. No.
5-Bromo-4-chloro-3-indolyl-myo-inositol 1-phosphate ammonium salt	38896-10MG-F 38896-50MG-F

## Substrates for Kallikrein

Name	Cat. No.
N-Benzoyl-Pro-Phe-Arg-p-nitroanilide hydrochloride	B2133-5MG B2133-25MG B2133-100MG

## Substrates for Lipase

Name	Cat. No.
4-Nitrophenyl dodecanoate	61716-1G 61716-5G
4-Nitrophenyl myristate	70124-1G

## Substrates for $\alpha$ -Mannosidase

Name	Cat. No.
4-Nitrophenyl $\alpha$ -D-mannopyranoside	N2127-25MG N2127-100MG N2127-250MG N2127-500MG N2127-1G N2127-5G

## Substrates for $\beta$ -D-Mannosidase

Name	Cat. No.
4-Nitrophenyl $\beta$ -D-mannopyranoside	N1268-25MG N1268-50MG N1268-100MG N1268-250MG

## Substrates for Mutanolysin

Name	Cat. No.
Mutanolysin Assay Substrate	M3440-1VL





## Substrates for Neuraminidase

Name	Cat. No.
5-Bromo-4-chloro-3-indolyl $\alpha$ -D-N-acetylneuraminic acid sodium salt	B4666-2MG B4666-10MG
2-O-(o-Nitrophenyl)- $\alpha$ -D-N-acetylneuraminic acid	N1266-1MG
2-O-(p-Nitrophenyl)- $\alpha$ -D-N-acetylneuraminic acid	N1516-1MG N1516-5MG

## Substrates for Peptidase

Name	Cat. No.
Arg-Pro p-nitroanilide acetate salt	A1204-10MG A1204-100MG

## Substrates for Phosphodiesterase

Name	Cat. No.
Thymidine 5'-monophosphate p-nitrophenyl ester sodium salt	T4510-25MG T4510-100MG T4510-250MG

## Substrates for Plasmin

Name	Cat. No.
N-(p-Tosyl)-Gly-Pro-Lys 4-nitroanilide acetate salt	T6140-5MG T6140-10MG T6140-25MG
D-Val-Leu-Lys 4-nitroanilide dihydrochloride	V0882-1MG V0882-5MG V0882-25MG

## Substrates for Protease

Name	Cat. No.
Azoalbumin	A2382-5G A2382-10G
Azocasein	A2765-1G A2765-5G A2765-10G A2765-25G
Azo dye-impregnated collagen	A4341-1G A4341-5G
N <sub>ε</sub> -Benzoyl-L-arginine 4-nitroanilide hydrochloride	B3279-100MG B3279-250MG B3279-1G
N <sub>ε</sub> -Benzoyl-L-arginine 4-nitroanilide hydrochloride	B3133-25MG B3133-100MG B3133-250MG B3133-1G
Hide-Remazol Brilliant Blue R	H6268-1G H6268-5G
N-Methoxysuccinyl-Ala-Ala-Pro-Val	M2897-10MG
N-Methoxysuccinyl-Phe-Leu-Phe-7-amido-4-trifluoromethylcoumarin	M4813-10MG
N-Methoxysuccinyl-Phe-Leu-Phe 4-methoxy-2-naphthylamide	M4938-10MG
N-Succinyl-Ala-Ala-Val-Ala p-nitroanilide	S7632-25MG
Val-Ala p-Nitroanilide acetate salt	V2503-25MG V2503-100MG
D-Val-Leu-Lys p-nitroanilide dihydrochloride	V7127-5MG V7127-25MG V7127-100MG
Z-Gly-Gly-Leu p-nitroanilide	C3022-25MG
Z-Gly-Pro-Arg p-nitroanilide acetate salt	C2276-25MG
Z-Val-Gly-Arg p-nitroanilide acetate salt	C7271-10MG C7271-25MG C7271-50MG

## Substrates for $\alpha$ -L-Rhamnosidase

Name	Cat. No.
4-Nitrophenyl $\alpha$ -L-rhamnopyranoside	N7763-100MG N7763-500MG

## Substrates for Sulfatase

Name	Cat. No.
Potassium 4-nitrophenyl sulfate	N3877-500MG N3877-1G N3877-5G

## Substrates for Thrombin

Name	Cat. No.
N-Benzoyl-Phe-Val-Arg-p-nitroanilide hydrochloride	B7632-5MG B7632-10MG B7632-50MG
Sar-Pro-Arg p-nitroanilide dihydrochloride	S9009-10MG S9009-25MG
N-(p-Tosyl)-Gly-Pro-Arg p-nitroanilide acetate salt	T1637-1MG T1637-5MG T1637-25MG T1637-100MG

## Substrates for Tissue Plasminogen Activator

Name	Cat. No.
Tissue plasminogen activator chromogenic substrate	T2943-25MG

## Substrates for Urokinase

Name	Cat. No.
N-Benzoyl-Val-Gly-Arg p-nitroanilide hydrochloride	B4758-5MG

## Substrates for Endo-1,4- $\beta$ -D-Xylanase

Name	Cat. No.
4-O-Methyl-D-glucurono-D-xylan dyed with Remazol brilliant blue R	66960-100MG 66960-500MG

## Substrates for $\alpha$ -Xylosidase

Name	Cat. No.
4-Nitrophenyl $\alpha$ -D-xylopyranoside	N1895-10MG N1895-50MG N1895-250MG

## Substrates for $\beta$ -Xylosidase

Name	Cat. No.
4-Nitrophenyl $\beta$ -D-xylopyranoside	N2132-500MG N2132-1G

# Enzyme Detection by Luminescence Methods

## Substrate for $\beta$ -D-Fucosidase

Name	Cat. No.
4-Methylumbelliferyl $\beta$ -D-fucoside	M5510-10MG M5510-25MG

## Substrate for Lysozyme

Name	Cat. No.
4-Methylumbelliferyl $\beta$ -D-N,N',N''-triacylchitotrioside	M5639-1MG M5639-5MG

## Substrates for N-Acetyl- $\beta$ -D-Galactosaminidase

Name	Cat. No.
4-Methylumbelliferyl N-acetyl- $\beta$ -D-galactosaminide	M9659-25MG M9659-100MG M9659-250MG M9659-1G

## Substrates for N-Acetyl- $\beta$ -D-Glucosaminidase

Name	Cat. No.
4-Methylumbelliferyl N-acetyl- $\beta$ -D-glucosaminide	69585-250MG 69585-1G

## Substrates for $\alpha$ -L-Arabinosidase

Name	Cat. No.
4-Methylumbelliferyl $\alpha$ -L-arabinofuranoside	M9519-10MG M9519-50MG

## Substrates for Cellulase

Name	Cat. No.
4-Methylumbelliferyl $\beta$ -D-cellobioside	M6018-100MG M6018-500MG

## Substrates for Chitinase

Name	Cat. No.
4-Methylumbelliferyl $\beta$ -D-N,N'-diacetylchitobioside hydrate	M9763-5MG M9763-10MG M9763-25MG
4-Methylumbelliferyl $\beta$ -D-N,N',N''-triacylchitotrioside	M5639-1MG M5639-5MG

## Substrates for Chymotrypsin

Name	Cat. No.
4-Methylumbelliferyl p-trimethylammoniocinnamate chloride	M4507-25MG M4507-100MG

## Substrates for Esterase

Name	Cat. No.
4-Methylumbelliferyl acetate	M0883-5G
4-Methylumbelliferyl butyrate	19362-1G 19362-5G

## Substrates for $\alpha$ -L-Fucosidase

Name	Cat. No.
4-Methylumbelliferyl $\alpha$ -L-fucopyranoside	M8527-1MG M8527-5MG M8527-10MG M8527-25MG M8527-100MG

## Substrates for $\alpha$ -Galactosidase

Name	Cat. No.
4-Methylumbelliferyl $\alpha$ -D-galactopyranoside	M7633-25MG M7633-100MG M7633-250MG

## Substrates for $\beta$ -Galactosidase

Name	Cat. No.
Fluorescein di( $\beta$ -D-galactopyranoside)	F2756-1MG F2756-5MG F2756-25MG
4-Methylumbelliferyl $\beta$ -D-galactopyranoside	M1633-250MG M1633-1G M1633-5G
4-Methylumbelliferyl $\beta$ -D-lactopyranoside	M2405-25MG M2405-100MG

## Substrates for $\alpha$ -Glucosidase

Name	Cat. No.
4-Methylumbelliferyl $\alpha$ -D-glucopyranoside	M9766-10MG M9766-25MG M9766-100MG M9766-250MG M9766-1G

## Substrates for $\beta$ -Glucosidase

Name	Cat. No.
4-Methylumbelliferyl $\beta$ -D-glucopyranoside	M3633-100MG M3633-250MG M3633-500MG M3633-1G M3633-5G

## Substrates for $\beta$ -Glucuronidase

Name	Cat. No.
4-Methylumbelliferyl $\beta$ -D-glucuronide hydrate	69602-250MG 69602-1G
4-Methylumbelliferyl $\beta$ -D-glucuronide hydrate	M5664

## Substrates for Lipase

Name	Cat. No.
Fluorescein diacetate	F7378-5G F7378-10G F7378-25G F7378-100G
Fluorescein dilaurate	46943-1G-F 46943-5G-F
Lipase Substrate	30058-10MG-F 30058-50MG-F
4-Methylumbelliferyl oleate	75164-25MG 75164-100MG

## Substrates for $\alpha$ -Mannosidase

Name	Cat. No.
4-Methylumbelliferyl $\alpha$ -D-mannopyranoside	M3657-10MG M3657-25MG M3657-100MG

## Substrates for $\beta$ -D-Mannosidase

Name	Cat. No.
4-Methylumbelliferyl $\beta$ -D-mannopyranoside	M0905-10MG M0905-25MG M0905-100MG M0905-250MG

## Substrates for Matrix Metalloproteinase

Name	Cat. No.
7-Methoxycoumarin-4-acetyl-Pro-Leu-Gly-Leu- $\beta$ -(2,4-dinitrophenylamino)Ala-Arg amide	M6412-1MG

## Substrates for Neuraminidase

Name	Cat. No.
2'-(4-Methylumbelliferyl)- $\alpha$ -D-N-acetylneuraminic acid sodium salt hydrate	M8639-1MG M8639-5MG M8639-25MG

## Substrates for $\alpha$ -L-Rhamnosidase

Name	Cat. No.
4-Methylumbelliferyl $\alpha$ -L-rhamnopyranoside	M8412-25MG M8412-100MG M8412-500MG

## Substrates for Sulfatase

Name	Cat. No.
4-Methylumbelliferyl sulfate potassium salt	M7133-500MG M7133-1G

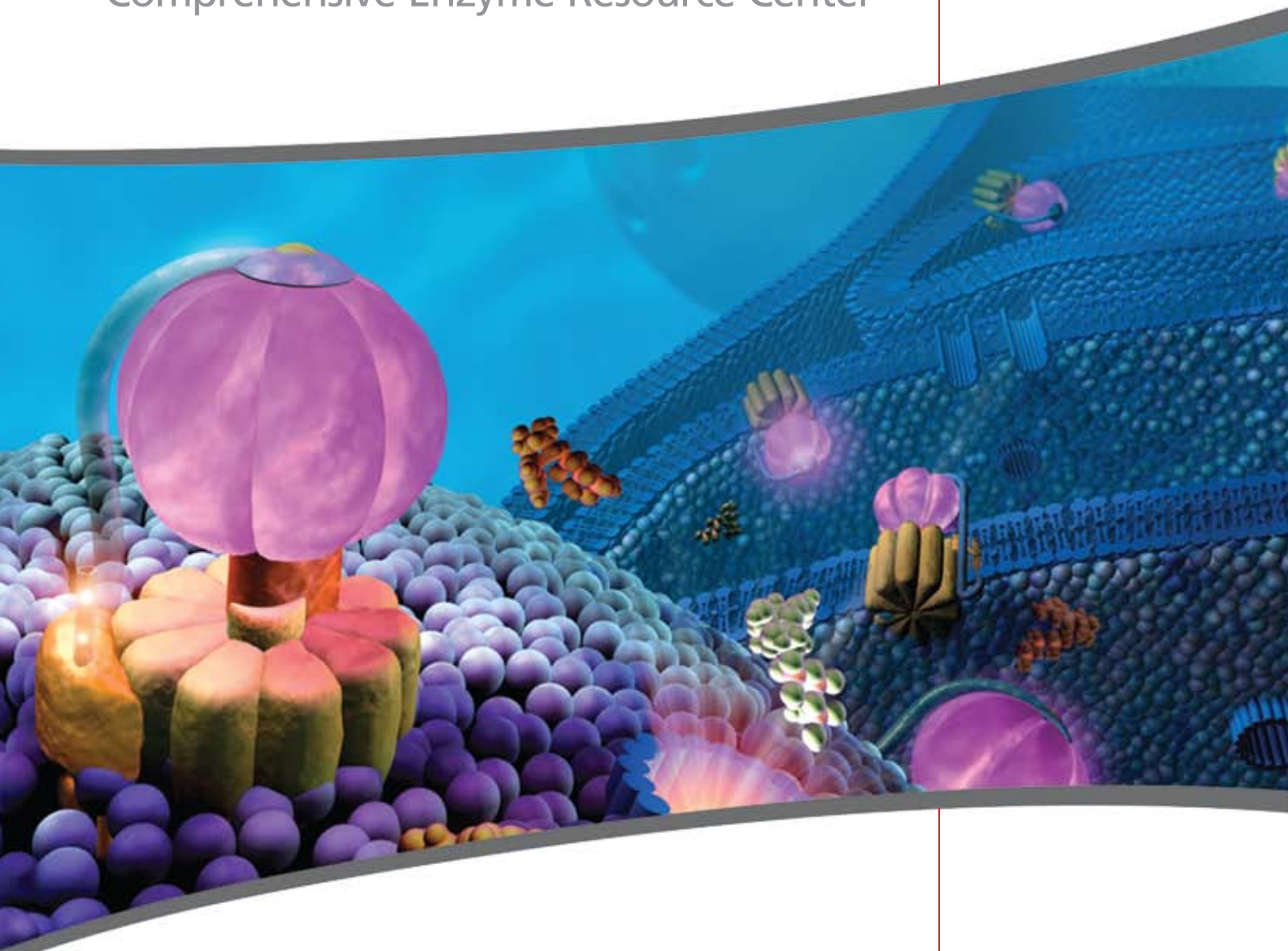
## Substrates for $\beta$ -Xylosidase

Name	Cat. No.
4-Methylumbelliferyl $\beta$ -D-xylopyranoside	M7008-25MG M7008-100MG M7008-250MG M7008-1G



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