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Lab & Production Materials



# petect the impossible

## Protein-Protein Interaction for Flow Cytometry

Traditional flow cytometry has been limited in the ability to detect protein interactions and low abundant proteins events — until now. We have combined Duolink<sup>®</sup> Proximity Ligation Assay (PLA), **SigmaAldrich.com/Duolink**, with flow cytometry in a convenient kit, making the analysis of proteinprotein interactions with flow cytometry readouts a reality. With the enhanced sensitivity of Duolink<sup>®</sup> flowPLA Kits, detection of low-abundant protein targets in cell populations is now possible. This easy-to-follow protocol works with any flow cytometry instrument to achieve publication-ready results.

The Duolink® flowPLA Starter Kits provide enough material to perform  ${\sim}40$  reactions, each with 100,000 cells in 100  $\mu L$  reaction volume.

### Duolink<sup>®</sup> flowPLA Starter and Detection Kits

Starter Kits include PLA probes and necessary reagents

Cat. No.	Description	Excitation (nm)	Excitation Laser Line (nm)*	Emission (nm)
DUO94102	Duolink flowPLA Starter Kit Mouse/Rabbit-Green	495	488	527
DUO94104	Duolink flowPLA Starter Kit-Mouse/Rabbit-Far Red	644	638, 640, 642	669
DUO94105	Duolink flowPLA Starter Kit Mouse/Rabbit-Violet	390	405	476
DUO94001	Duolink <sup>®</sup> flowPLA Detection - Red	594	532, 561	624
DUO94002	Duolink <sup>®</sup> flowPLA Detection - Green	495	488	527
DUO94003	Duolink <sup>®</sup> flowPLA Detection - Orange	554	5,32,561	579
DUO94004	Duolink <sup>®</sup> flowPLA Detection - FarRed	644	638, 640, 642	669
DUO94005	Duolink <sup>®</sup> flowPLA Detection - Violet	390	405	476

\*The excitation laser line represents commonly used lasers that excite the fluorophore. It does not necessarily reflect the lasers available for each instrument.

It is recommended that Duolink flowPLA Compensation Beads be used when performing a multiplexed experiment to save time, reagent cost and precious sample.

#### **Duolink flowPLA Compensation Beads**

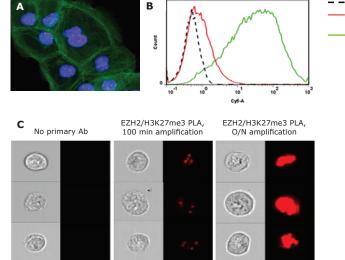
Cat. No.	Description
DUO84011	Red
DUO84012	Green
DUO84013	Orange
DUO84014	Far Red
DUO84015	Violet
DUO84010	Unstained





## Protein-Protein Interaction for Flow Cytometry

Figure 1. Increased amplification time during Duolink® PLA can aid in the detection of low-abundant protein targets by flow cytometry. Duolink® PLA was performed to detect the trimethylation of lysine 27 on histone 3 (H3K27me3) mediated by EZH2. A) Few PLA signals (red) in the nuclei (blue) of DU145 cells were detected by fluorescence microscopy after 100 min amplification. FITC-Phalloidin-stained actin (green) was used as a counterstain. B) Extended amplification times enhanced the detection of low-abundant protein events, such as EZH2-H3K27me3 interactions, by conventional flow cytometry.
C) Combining Duolink<sup>®</sup> PLA with imaging flow cytometry allows localization of proteins or protein events (interactions or modifications) in large cell populations.



 No primary Ab
EZH2/H3K27me3 PLA, 100 min amplification
EZH2/H3K27me3 PLA, Overnight amplification

#### For more information, visit SigmaAldrich.com/flowpla

To place an order or receive technical assistance in the U.S. and Canada, call toll-free 1-800-645-5476 For other countries across Europe and the world, please visit: **EMDMillipore.com/offices** For Technical Service, please visit: **EMDMillipore.com/techservice**  MilliporeSigma 400 Summit Drive Burlington, MA 01803

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