

For life science research only.
Not for use in diagnostic procedures.



Insulin, human recombinant (yeast)

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Cat. No. 11 376 497 001 100 mg

Store lyophilizate at +2 to +8°C.

1.	General Information	3
1.1.	Contents	3
1.2.	Storage and Stability	3
	Storage Conditions (Product)	3
	Storage Conditions (Working Solution).....	3
	Reconstitution	3
1.3.	Additional Equipment and Reagent required	3
1.4.	Application	3
2.	How to Use this Product	4
2.1.	Before you Begin	4
	General Considerations.....	4
	Primary structure	4
	Working Solution.....	4
2.2.	Protocols	4
	Determination of insulin activity	4
2.3.	Parameters	5
	Biological Activity	5
	Molecular Weight	5
	Purity.....	5
	Specific Activity	5
	Specificity	5
	Unit Definition.....	5
	EC ₅₀ definition	5
	Working Concentration.....	5
3.	Additional Information on this Product	6
3.1.	Test Principle	6
	Preparation.....	6
	How this product works.....	6
	Stimulation of cell growth.....	6
4.	Supplementary Information	7
4.1.	Conventions.....	7
4.2.	Changes to previous version.....	7
4.3.	Ordering Information.....	7
4.4.	Trademarks.....	8
4.5.	License Disclaimer.....	8
4.6.	Regulatory Disclaimer.....	8
4.7.	Safety Data Sheet	8
4.8.	Contact and Support.....	8

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Insulin, human	<ul style="list-style-type: none"> Lyophilized from a hydrochloric acid solution, pH 2.3 (crystalline insulin). Filtered through 0.2 µm pore size membrane prior to lyophilization. 	1 bottle, 100 mg

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the lyophilizate is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	Insulin, human	Store at +2 to +8°C.

Storage Conditions (Working Solution)

Store the reconstituted, undiluted solution at –15 to –25°C.

Reconstitution

Reconstitute lyophilizate in double-distilled water (final concentration 10 mg/ml). It can be further diluted with PBS (phosphate buffered saline) or medium containing 1 mg/ml (0.1%) BSA (bovine serum albumin) or HSA (human serum albumin), or 1 to 10% serum.

1.3. Additional Equipment and Reagent required

For reconstitution of lyophilizate and dilution

- Double-distilled water
- PBS (phosphate buffered saline)
- Medium containing 1 mg/ml (0.1%) BSA or HSA, or 1 to 10% serum

For determination of insulin activity

- Culture medium, such as DMEM/F12 (1:1) containing 15 mM HEPES, 2 mM L-glutamine, 50 µg/ml gentamycin, 10 µg/ml Transferrin*, 10 µg/ml ovalbumin, 1 µM dexamethasone, and 20 ng/ml Basic Fibroblast Growth Factor human (hbFGF)*.
- Poly-D-lysine dissolved in sterile-filtered PBS or double-distilled water (1 mg/ml) and diluted with sterile-filtered PBS or double-distilled water to a final concentration of 0.1 mg/ml.
- Trypsin*

1.4. Application

Insulin shows a broad range of activities on a variety of somatic cells. Use recombinant, human insulin to stimulate growth and proliferation of cultured cells and to investigate insulin activity on sensitive cells used in medical research studies. It is also a component of serum-free media formulations for most primary cells and cell lines.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Primary structure

Recombinant, human insulin consists of two poly-peptide chains (A-chain: 21 amino acids, B-chain: 30 amino acids) connected by two disulphide bridges and is identical to natural, human insulin.

Working Solution

Dissolve Insulin, human, recombinant, in double-distilled water to a final concentration of 10 mg/ml.

i The concentration of insulin required for stimulation of cell growth in almost all cases is extraordinarily high compared with the physiological concentration. Insulin may be mimicking insulin-like growth factors (IGFs, somatomedins) for some cell lines, and high insulin concentrations may be necessary in order to occupy receptors which have a high affinity for IGFs and a lower affinity for insulin.

2.2. Protocols

Determination of insulin activity

Use the following protocol to determine the activity on sensitive cells in serum-free cell culture (insulin proliferation assay).

- 1 Seed cells, such as Balb/c 3T3 fibroblasts (clone A31) at a concentration of 1.0×10^4 cells/ml into 35 mm cell culture plates (3 ml/dish), coated with 0.1 mg/ml poly-D-lysine in culture medium without insulin.

- 2 Add 1.0 ml culture medium containing various amounts of insulin (final concentration from 10 ng/ml to 10 µg/ml) to each culture plate.

- 3 Incubate the cultures for 5 to 6 days at +37°C and 5% CO₂.

- 4 After this incubation period, trypsinize the cells and determine cell number.

2.3. Parameters

Biological Activity

<250 ng/ml, at least the same specific activity (EC_{50}) compared to the indicated standard is guaranteed.

Molecular Weight

5,700 Da

Purity

>98% pure as determined by SDS-PAGE.

Specific Activity

>26 U/mg

Specificity

Insulin is effective on most mammalian cells.

Unit Definition

EC_{50} definition

The concentration of human insulin that is required to support half-maximal stimulation of cell proliferation (MTT cleavage) with 3T3 (A31) cells.

Working Concentration

Concentration for serum-free cell culture is 1 to 10 μ g/ml.

3. Additional Information on this Product

3.1. Test Principle

Preparation

Recombinant, human insulin is produced in yeast and purified by standard chromatographic techniques.

How this product works

Insulin stimulates the growth and proliferation of a variety of somatic cells in culture, and evidence suggests that insulin is also an important regulator of growth *in vivo*. *In vitro*, insulin interacts synergistically with other hormones and growth factors, such as PDGF, EGF, and FGF in stimulation of cells that have been arrested in G1 phase by deprivation of serum. In addition, insulin is required by most cells for optimal long-term growth in hormone-supplemented, serum-free media.

Stimulation of cell growth

Insulin initiates its action by binding to a glycoprotein receptor (insulin receptor) on the surface of the cell. Binding to this receptor generates a signal that eventually results in insulin's action on glucose, lipid, and protein metabolism. The growth-promoting effects of insulin appear to occur through activation of receptors for the family of related insulin-like growth factors (IGFs). In cell culture, insulin is a component of serum-free media formulations for all primary cells and cell lines examined so far. In addition to the stimulation of cell growth, classical insulin responses, such as increased fatty acid and glycogen synthesis are seen in serum-free medium.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols

 *Information Note: Additional information about the current topic or procedure.*

 **Important Note: Information critical to the success of the current procedure or use of the product.**

① ② ③ etc. Stages in a process that usually occur in the order listed.

① ② ③ etc. Steps in a procedure that must be performed in the order listed.

* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.

Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Trypsin recombinant, Proteomics Grade	4 x 25 µg	03 708 985 001
	4 x 100 µg	03 708 969 001
Transferrin	20 ml, 30 mg/ml	10 652 202 001

4. Supplementary Information

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

