

## PRODUCT INFORMATION

### Collagenase NB 1 Premium Grade

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**General** Collagenases from *Clostridium histolyticum* are proteolytic enzymes that cleave peptide bonds in the triple helical collagen molecule of human or animal tissue *in situ*.

For this reason collagenases are widely used for isolation of various cell types by tissue dissociation.

**Description** Collagenase NB 1 Premium Grade is chromatographically highly purified; therefore it contains a very high collagenolytic activity. It is largely free from additional enzymatic activities like clostripain, trypsin-like activity and neutral protease, as well as endotoxins.

<b>Specification</b>	Collagenase activity	≥ 3.00 U/mg (PZ acc. to Wunsch)
		≥ 2000 U/vial (PZ acc. to Wunsch)
	Neutral protease activity	≤ 0.05 U/mg (DMC)
	Trypsin-like activity	status
	Clostripain, native state	status
	Endotoxin	≤ 10.0 EU/mg

**Application** Collagenase NB 1 Premium Grade is, mostly in combination with Neutral Protease NB (Cat. No. S3030112), suitable for cell isolation from several tissue types.

If a GMP conform product is required, Collagenase NB 1 GMP Grade (Cat. No. N0002937) is recommended. This product has comparable enzymatic activities to Collagenase NB 1 Premium Grade.

**Storage conditions** Collagenase NB 1 Premium Grade is provided as a lyophilized powder. It should be stored at +2 to +8 °C in a dry environment. Under these conditions the product is stable until the minimum shelf life stated on the certificate of analysis if repeated opening and closing of the vial is avoided.

For storage of solutions please refer to “Stock solution”.

**Documents** For each lot a specific certificate of analysis is provided. A certificate of origin is available.

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### Instructions for use:

**General** Collagenase NB 1 Premium Grade is, in combination with Neutral Protease NB, especially suited for isolation of islets of Langerhans from human pancreas, and also for cell isolation from several other human or animal tissues.

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**Tissue dissociation** Recommended starting concentrations for isolation of islets of Langerhans from human pancreas:

Collagenase NB 1 Premium Grade: 15 – 20 PZ U/g tissue  
Neutral Protease NB: 0.8 – 1.5 DMC U/g tissue

Collagenase NB 1 Premium Grade is provided in vials with not less than 2000 PZ U which is usually sufficient for dissociation of one human pancreas.

In general, the appropriate collagenase concentration depends on tissue type and origin as well as on the isolation procedure.

Collagenase activity is at an optimum at 37 °C and pH 7.4.

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**Stock solution** A stock solution of Collagenase NB 1 Premium Grade is prepared by dissolving the enzyme in buffer. The enzyme solution must be constantly stored on ice.

Since collagenase depends on calcium, it is recommended to use a buffer with  $\geq 2$  mM  $\text{Ca}^{2+}$ . Absolutely no calcium chelating agents (e.g. EDTA) should be present at all.

Reconstituted Collagenase NB 1 Premium Grade can be 0.22  $\mu\text{m}$  filtered, aliquoted and stored at -20 °C. Aliquots are stable for 1 year if repeated freezing and thawing is avoided.

For 0.22  $\mu\text{m}$  filtration filters with low protein-binding properties (e.g. cellulose acetate, PVDF, or PES) are recommended.

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**Working solution** To prepare a working solution, the stock solution is diluted with buffer to achieve the required collagenase concentration. The working solution must be constantly stored on ice until use.

If Collagenase NB 1 Premium Grade solution is mixed with Neutral Protease NB solution, the blend should be used immediately.

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**Inactivation and inhibitors** The dissociation process can be reduced, e.g. by cooling down or dilution of the enzyme solution.

Collagenase is reversibly inactivated at high pH values and irreversibly inactivated at low pH values. Inhibitors of collagenase include cysteine or chelating agents like EDTA.

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**Important note** Collagenase NB 1 Premium Grade is not intended for direct application in humans.