

## PRODUCT INFORMATION

**Collagenase NB 1 GMP Grade**

**Cat. No. N0002937**

**General** Collagenases from *Clostridium histolyticum* are proteolytic enzymes that cleave peptide bonds in the triple helical collagen molecules 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 GMP Grade is manufactured according to GMP guidelines. TSE safety of the manufacturing process was certified by the EDQM. A virus validation study and stability studies according to ICH guidelines were performed.

Collagenase NB 1 GMP 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	≥ 3.000 U/mg (PZ acc. to Wünsch) ≥ 2000 U/vial (PZ acc. to Wünsch)
	Neutral protease activity	≥ 0.050 U/mg (DMC)
	Trypsin-like activity	status
	Clostripain, native state	status
	TAMC	≤ 10/vial
	TYMC	≤ 10/vial
	Bacterial endotoxins	≤ 10.0 EU/mg

**Application** Collagenase NB 1 GMP Grade is, mostly in combination with Neutral Protease NB GMP Grade (Cat. No. N0002936), suitable for cell isolation from several tissue types intended for clinical applications.

If a research product is required, Collagenase NB 1 Premium Grade (Cat. No. S1745503) is recommended.

**Storage conditions** Collagenase NB 1 GMP Grade is available 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 expiry date 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 customer-specific certificate of analysis is provided. An EDQM certificate on TSE safety, a summary of the virus safety study, and stability summary reports are available.

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## Instructions for use:

**General** Collagenase NB 1 GMP Grade is, in combination with Neutral Protease NB GMP Grade, especially suited for isolation of islets of Langerhans from human pancreas intended for transplantation into humans, and also for cell isolation from several other human tissues.

**Tissue dissociation** Recommended starting concentrations for isolation of islets of Langerhans from human pancreas:  
Collagenase NB 1 GMP Grade: 15 – 20 PZ U/g tissue  
Neutral Protease NB GMP Grade: 0.8 – 1.5 DMC U/g tissue  
Collagenase NB 1 GMP 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.

**Stock solution** A stock solution of Collagenase NB 1 GMP 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 GMP 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.

**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 GMP Grade solution is mixed with Neutral Protease NB GMP Grade solution, the blend should be used immediately.

**Inactivation and inhibitors** The dissociation process can be reduced, e.g. by cooling down or diluting 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.

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