

# PRODUCT INFORMATION



**Collagenase NB 1 GMP Grade**  
**Neutral Protease NB GMP Grade**

**Cat. No. 17452**  
**Cat. No. 30303**

## Product Description:

**General** Collagenase NB 1 GMP Grade and Neutral Protease NB GMP Grade are designed for isolation of islets of Langerhans which are intended for transplantation into humans. The production complies with Annex 18 to the EU-Guide to Good Manufacturing Practices, GMP for Active Pharmaceutical Ingredients, and requirements for reagents used in cell production of the European Pharmacopoeia, as far as applicable. The enzymes are produced by a carefully selected strain of *Clostridium histolyticum*. Chromatographic purification yields highly purified enzymes with a constant ratio of class I and II collagenases.

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### Specification **Collagenase NB 1 GMP Grade:**

Collagenase activity	≥ 3.0 U/mg* (PZ acc. to Wunsch)
Collagenase activity	≥ 2,000 U/vial* (PZ acc. to Wunsch)
Neutral protease activity	≤ 0.05 U/mg (DMC)
Trypsin-like activities	≤ 0.5 U/mg (BAEE)
Purity	Must comply
Colony forming units	≤ 10 CFU/Vial
Endotoxin	≤ 10 EU/mg
Abnormal Toxicity	Must comply

### **Neutral Protease NB GMP Grade:**

Neutral protease activity	≥ 0.5 U/mg* (DMC)
Neutral protease activity	≥ 100 U/vial* (DMC)
Purity	Must comply
Colony forming units	≤ 10 CFU/Vial
Endotoxin	≤ 100 EU/mg
Abnormal Toxicity	Must comply

**\* For enzymatic activity and total enzymatic activity per vial please refer to Certificate of Analysis.**

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**Application** Collagenase NB 1 GMP Grade and Neutral Protease NB GMP Grade are designed for dissociation of human pancreatic tissue for isolation of islets of Langerhans for transplantation into humans. The enzymes are not intended for use in humans. Responsibility for clinical use and methods to isolate, purify and transplant islets lies solely with the providing physician/researcher.

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**Storage conditions** Both enzymes are provided separately as lyophilized powders and should be stored **in a dry state**:  
Collagenase NB 1 GMP Grade at +2 to +8 °C;  
Neutral Protease NB GMP Grade at +2 to +8 °C.

## Collagenase NB 1 GMP Grade Neutral Protease NB GMP Grade

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### Instructions for use:

<b>General</b>	Several experimental procedures have been described for isolation of islets of Langerhans in literature. However, some features have to be considered when using Collagenase NB 1 GMP Grade and Neutral Protease NB GMP Grade.
<b>Required enzymatic activity</b>	Collagenase NB 1 GMP Grade is provided in vials containing $\geq 2,000$ PZ units which is mostly sufficient for dissociation of one human pancreas. Experimental studies revealed a recommended activity of about 20 PZ units per gram cleaned human organ (fat and membranes dissected from the surface) <sup>1</sup> . Due to the low neutral protease level in Collagenase NB 1 GMP Grade, <b>it is necessary to add Neutral Protease NB GMP Grade</b> to the enzyme solution for dissociation <sup>2</sup> . Neutral protease activity should be added in the range of 0.8 up to 1.5 DMC units per gram human pancreas for the digestion. The dosage of Neutral Protease NB GMP Grade depends on the condition of the pancreas in terms of fibrotic or fatty degeneration, time of ischemia etc. For less easily digestible organs a higher amount of Neutral Protease NB GMP Grade has to be added.
<b>Stock solution</b>	Stock solution of Collagenase NB 1 GMP Grade can be prepared in buffer solution (e.g. 25 ml HBSS) containing $\geq 3.1$ mM $\text{CaCl}_2$ by agitating <b>at 4 °C</b> for 15 to 30 min. Neutral Protease NB GMP Grade should be dissolved separately in <b>water</b> (e.g. 5 ml) <b>at 4 °C</b> . For calculation of the enzyme concentration please consider the total enzymatic activity per vial as stated on the Certificate of Analysis. The solutions should be prepared directly before use and should not be stored for more than 2 hours. If sterile filtration is desired a sterile filter with low protein binding properties (e.g. PES, PVDF) should be used with the filter being rinsed with an additional small volume of buffer solution before and after filtration.
<b>Working solution</b>	The working solution is prepared by mixing the two cold enzyme stock solutions with cold dissociation buffer (containing $\geq 3.1$ mM $\text{CaCl}_2$ ) to obtain the desired volume for the tissue dissociation. After mixing, the working solution should be applied to the organ immediately.

<sup>1</sup> Bucher et al; *Transplantation*, **79**, 91-97 (2005)

<sup>2</sup> Brandhorst et al; *Transplant Proc.*, **37(1)**, 241-242 (2005)