

PRODUCT INFORMATION

SERVA
Electrophoresis



Collagenase NB 1 Premium Grade Neutral Protease NB

Cat. No. 17455
Cat. No. 30301

Product Description:

General Collagenase NB 1 and Neutral Protease NB are used for dissociation of pancreatic tissues of different species (e.g. human, rat, pig) for isolation of islets of Langerhans. The enzymes are produced by a carefully selected strain of *Clostridium histolyticum* and are non-toxic according to the requirements of the European Pharmacopoeial Test for Abnormal Toxicity, General Safety Test. Chromatographic purification yields highly purified enzymes with extremely low concentrations of endotoxins and Collagenase NB 1 with a constant ratio of class I and II collagenases.

Specification **Collagenase NB 1 Premium 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)
Endotoxin	≤ 10 EU/mg

Neutral Protease NB:

Neutral protease activity: ≥ 0.1 U/mg* (DMC)

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

Application Collagenase NB 1 and Neutral Protease NB are designed for dissociation of pancreatic tissues of different species (e.g. human, rat, pig) for isolation of islets of Langerhans, which are used for research purposes. 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.

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

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Instructions for use:

General	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 and Neutral Protease NB.
Required enzymatic activity	Collagenase NB 1 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 or rat organ or 15-18 PZ units per gram cleaned porcine organ (fat and membranes dissected from the surface) ^{1, 2} . Due to the low neutral protease level in Collagenase NB 1, it is necessary to add Neutral Protease NB to the enzyme solution for dissociation ³ . Neutral protease activity should be added in the range of 0.8 up to 1.5 DMC units per gram pancreas for the digestion. The dosage of Neutral Protease NB 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 has to be added.
Stock solution	Stock solution of Collagenase NB 1 can be prepared in buffer solutions (e.g. 25 ml HBSS), containing ≥ 3.1 mM CaCl_2 by agitating at 4 °C for 15 to 30 min. Neutral Protease NB should be dissolved separately in water (e.g. 5 ml) at 4 °C. For calculation of the enzyme concentration of Collagenase NB 1 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.
Working solution	The working solution is prepared by mixing the two cold enzyme stock solutions with cold dissociation buffer (containing ≥ 3.1 mM CaCl_2) to obtain the desired volume for the tissue dissociation. After mixing, the working solution should be applied to the organ immediately.

¹ Bucher et al; *Transplantation*, **79**, 91-97 (2005)

² Brandhorst et al; *Transplantation*, **79**, 38-42 (2005)

³ Brandhorst et al; *Transplant Proc.*, **37(1)**, 241-242 (2005)

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