

## PRODUCT INFORMATION

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### Collagenase NB 5 Sterile Grade

Cat. No. 17459

#### Product Description:

**General** Collagenases from *Clostridium histolyticum* are proteolytic enzymes that are able to cleave peptide bonds in the triple helical collagen molecule of human or animal tissue *in situ*. The collagenases are divided into class I and class II collagenase isoforms on the basis of their activities towards synthetic peptides. Both collagenase isoforms act on triple helical type I, II, III and IV collagens, but in slightly different modes of action. Collagenase NB 5 Sterile Grade contains collagenase classes I and II as well as a balanced ratio of other proteolytic activities such as neutral protease and clostripain. Therefore Collagenase NB 5 is a mild and effective enzyme producing high yields of viable cells. Collagenase NB 5 is sterile according to European requirements for medicinal products (Pharm. Eur.) and thus it is ready for use.

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<b>Specification</b>	Collagenase activity	≥ 0.1 U/mg (PZ units acc. to Wunsch)
	Sterility (Ph. Eur.)	Must comply

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**Application** Collagenase NB 5 Sterile Grade is designed for dissociation of different tissues to isolate a broad variety of cells, e.g. adipocytes, chondrocytes, endo- and epithelial cells, islets, osteoblasts, different kind of stem and progenitor cells from various species as well as for stem cell passaging. The use of Collagenase NB 5 Sterile Grade is intended for research purposes only and is not intended for use in humans.

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**Storage conditions** Collagenase NB 5 Sterile Grade is provided as a lyophilized powder and should be stored **in a dry state** at +2 to +8 °C. The expiry date is printed on the label on each vial. Dissolved enzymes can be aliquoted and stored at -20°C for one year. Repeated freezing and thawing should be avoided.

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#### Instructions for use

**General** For isolation of each cell type a specific isolation procedure has to be applied to receive optimal yields of viable cells. For many cell types detailed protocol information is available at [www.crescentchemical.com](http://www.crescentchemical.com)

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**Stock solution** Collagenase NB 5 Sterile Grade easily dissolves in a concentration of up to 150 mg/mL in all buffers which are generally used for cell isolation. Keep the enzyme solution on ice. As collagenase and some of the secondary proteases require calcium both for full catalytic activity 2 mM Ca<sup>2+</sup> is recommended, and no calcium chelating agents (e.g. EDTA) should be present. The enzyme is reversibly inactivated at high pH values and irreversibly inactivated at low pH values. pH should be physiologically appropriate for the cells to be isolated (~ pH 7.4). Collagenase NB 5 is pharmaceutical sterile therefore no sterile filtration is necessary if sterile buffers and reagents are used.

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**Working solution** Stock solution is diluted with dissociation buffer to achieve the required enzymatic activity (see below). Dissociation can be terminated by buffer dilution and system cooling.



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**Required enzymatic activity**

For establishing of the procedure the following concentrations of Collagenase NB 5 Sterile Grade can be used as starting point:

Adipocytes (human, mouse)	0.20 - 0.30 PZ U/mL
Chondrocytes (human, mouse, bovine)	0.20 - 0.40 PZ U/mL
Chromaffin cells (bovine, rat)	0.30 - 0.50 PZ U/mL
Endometrial cells (human)	0.20 - 0.30 PZ U/mL
Endothelial cells (HUVEC)	0.50 - 0.70 PZ U/mL
Endothelial cells (HDMEC)	0.15 - 0.25 PZ U/mL
Epithelial cells (mouse)	0.34 PZ U/mL
Islets (neonatal pigs)	0.17 - 0.20 PZ U/mL
Lung cells (mouse)	0.20 PZ U/mL
Neurons (human, rodent)	0.15 - 0.25 PZ U/mL
Osteoblasts (mouse)	0.15 - 0.25 PZ U/mL
Schwann cells (rodent)	0.20 - 0.30 PZ U/mL
Stem cells (human, Wharton's jelly)	0.13 - 0.15 PZ U/mL
ADSC / preadipocytes (human, mouse)	0.20 - 0.30 PZ U/mL
Stem cell passaging (hESC)	0.15 PZ-U/mL

If digestion is incomplete or if cells are damaged, collagenase concentration, time or temperature should be adjusted. Within a range from 10 °C to 37 °C an increase in temperature of 10 °C results in approximate doubling of the collagenase PZ activity.

For further information concerning a specific application please contact our collagenase team at [crescent@creschem.com](mailto:crescent@creschem.com) or visit our web site at [www.creschem.com](http://www.creschem.com).