

## PRODUCT INFORMATION

### Collagenase NB 4 Standard 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 4 Standard Grade is a crude collagenase that contains collagenolytic and additional enzymatic activities including clostripain and neutral protease.

The balanced ratio of these activities ensures gentle and efficient tissue dissociation.

**Specification** Collagenase activity  $\geq 0.10$  U/mg (PZ acc. to Wunsch)

**Application** Collagenase NB 4 Standard Grade is suitable for dissociation of a broad variety of tissue types.

If a sterile/GMP conforming product is required, Collagenase NB 5 Sterile Grade (Cat. No. N0002778) or Collagenase NB 6 GMP Grade (Cat. No. N0002779), are recommended. Both products have comparable enzymatic activities to Collagenase NB 4 Standard Grade.

**Storage conditions** Collagenase NB 4 Standard 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.

**Product size**

Product	Cat.No.	Size (g)
Collagenase NB 4 Standard Grade	S1745402	0.5
	S1745401	1
	S1745403	5

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#### Instructions for use:

**General** Collagenase NB 4 Standard Grade is suitable for isolation of a broad variety of cells from human and animal tissues. Tissue types include adipose tissue, cartilage, skin, placenta, umbilical cord tissue, and lung. It can also be applied in cell culture for passaging, e.g. of embryonic stem cells.

**Tissue dissociation** Recommended starting concentrations for selected applications:  
 Adipose tissue (human, rodent): 0.2 – 0.3 PZ U/ml  
 Cartilage (human, rodent): 0.3 – 0.4 PZ U/ml

In general, the appropriate collagenase concentration depends on tissue type and origin as well as on the isolation procedure. Further protocol information for dissociation of several tissue types is available.

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

**Stock solution** Collagenase NB 4 Standard Grade dissolves at a concentration of up to 150 mg/ml in all buffers which are commonly used for cell isolation. The enzyme solution must be constantly stored on ice.

Since collagenase and some of the secondary proteases depend 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 4 Standard 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 stored on ice until use.

**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.

**Important note** Collagenase NB 4 Standard Grade is not intended for direct application in humans.

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