

## PRODUCT INFORMATION

**Collagenase NB 5 Sterile Grade**

**Cat. No. N0002778**

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<b>General</b>	<p>Collagenases from <i>Clostridium histolyticum</i> are proteolytic enzymes that cleave peptide bonds in the triple helical collagen molecules of human or animal tissue <i>in situ</i>.</p> <p>For this reason collagenases are widely used for isolation of various cell types by tissue dissociation.</p>				
<b>Description</b>	<p>Collagenase NB 5 Sterile Grade is sterile according to European Pharmacopoeia.</p> <p>Collagenase NB 5 Sterile 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.</p>				
<b>Specification</b>	<table><tr><td>Collagenase activity</td><td>≥ 0.10 U/mg (PZ acc. to Wunsch)</td></tr><tr><td>Sterility</td><td>must comply</td></tr></table>	Collagenase activity	≥ 0.10 U/mg (PZ acc. to Wunsch)	Sterility	must comply
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<b>Application</b>	<p>Collagenase NB 5 Sterile Grade is suitable for dissociation of a broad variety of tissue types.</p> <p>If a research or GMP conforming product is required, Collagenase NB 4 Standard Grade (Cat. No. S1745401) or Collagenase NB 6 GMP Grade (Cat. No. N0002779), respectively, are recommended. Both products have comparable enzymatic activities to Collagenase NB 5 Sterile Grade.</p>				
<b>Storage conditions</b>	<p>Collagenase NB 5 Sterile 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 minimum shelf life stated on the certificate of analysis if repeated opening and closing of the vial is avoided.</p> <p>For storage of solutions please refer to “Stock solution”.</p>				
<b>Documents</b>	<p>For each lot a specific certificate of analysis is provided. A certificate of origin is available.</p>				

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## Instructions for use:

**General** Collagenase NB 5 Sterile Grade is suitable for isolation of a broad variety of cells from human or animal tissues. Tissue types include adipose tissue, cartilage, skin, placenta, and umbilical cord tissue. 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 at [www.nordmark-biochemicals.com](http://www.nordmark-biochemicals.com).

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

**Stock solution** Collagenase NB 5 Sterile 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 5 Sterile Grade can be aliquoted and stored at -20 °C. Aliquots are stable for 1 year if repeated freezing and thawing is avoided.

Collagenase NB 5 Sterile Grade is sterile according to Ph. Eur. Therefore, 0.22  $\mu\text{m}$  filtration is not necessary if sterile equipment and buffers are used. If 0.22  $\mu\text{m}$  filtration is required, 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.

**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 5 Sterile Grade is not intended for direct application in humans.