

## PRODUCT INFORMATION

**Collagenase AF-1 GMP Grade ( $\geq 150$  U)**

**Cat. No. N0003855**

**Introduction** Collagenases from *Clostridium histolyticum* are proteolytic enzymes that cleave peptide bonds in the triple helical collagen molecules 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 AF-1 GMP Grade is chromatographically highly purified leading to a very high collagenolytic activity. It is largely free from additional enzymatic activities like clostripain, trypsin-like activities and neutral protease, as well as endotoxins.

Collagenase AF-1 GMP Grade is manufactured according to GMP guidelines using a production process completely free of animal-based components. Thus, the introduction of any potential animal-derived pathogen is excluded by design.

The absence of any animal-derived ingredients and thorough microbial analysis provide the highest possible safety.

**Specification**

Collagenase activity	$\geq 3.000$ U/mg (PZ acc. to Wunsch)
	$\geq 150$ U/vial (PZ acc. to Wunsch)
Neutral protease activity	$\leq 0.050$ U/mg (DMC)
Trypsin-like activities	status
Clostripain, native state	status
TAMC	$\leq 10$ /vial
TYMC	$\leq 10$ /vial
Bacterial endotoxins	$\leq 10.0$ EU/mg

**Application** Collagenase AF-1 GMP Grade is suitable for cell isolation from several tissue types intended for clinical applications.

For larger requirements, Collagenase AF-1 GMP Grade is also available in a pack size of  $\geq 2000$  PZ U per vial (Cat. No. N0003554).

Collagenase AF-1 GMP Grade is often used in combination with Neutral Protease AF GMP Grade (Cat. No. N0003553).

**Storage conditions** Collagenase AF-1 GMP 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 date stated on the certificate of analysis if repeated opening and closing of the vial is avoided.

**Documents** A certificate of analysis is provided for each lot.

For additional documentation (e.g. stability data, GMP certificate, certificate of origin, etc.), please contact our product management team at [collagenase@nordmark-biochemicals.com](mailto:collagenase@nordmark-biochemicals.com)

**Product size**

Product	Cat. No.	Size (PZ U)
Collagenase AF-1 GMP Grade	N0003855	$\geq 150$
	N0003554	$\geq 2000$

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## Instructions for use:

**General** Collagenase AF-1 GMP Grade, often in combination with Neutral Protease AF GMP Grade (Cat. No. N0003553), is suitable for the dissociation of sensitive cells from several tissues, such as liver, pancreas and tumor. In addition, it has been shown to be highly effective for the isolation of the stromal vascular fraction (SVF) from adipose tissue.

**Tissue dissociation** Recommended final concentrations for isolation of SVF from human lipoaspirate:

Collagenase AF-1 GMP Grade: 0.2 PZ U/ml  
Neutral Protease AF GMP Grade: 0.1 DMC U/ml

Collagenase AF-1 GMP Grade is provided in vials with  $\geq 150$  PZ U which is usually sufficient for approx. 800 ml lipoaspirate.

The appropriate collagenase concentration depends on the tissue type and origin as well as on the applied isolation procedure.

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

If you have more questions concerning your application, you are welcome to contact the product management team at:  
collagenase@nordmark-biochemicals.com

**Stock solution** A stock solution of Collagenase AF-1 GMP Grade can be prepared by dissolving the enzyme in buffer. The enzyme solution should be constantly stored on ice.

Since collagenase depends on calcium, absolutely no calcium chelating agents (e.g. EDTA) should be present at all. A buffer with  $\geq 2$  mM  $\text{Ca}^{2+}$  is recommended to be used.

Reconstituted Collagenase AF-1 GMP Grade can be filtered, aliquoted and stored at -20 °C for up to 1 year. Repeated freezing and thawing should be 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 to achieve the desired collagenase concentration.

The working solution should be constantly stored on ice until use.

If Collagenase AF-1 GMP Grade solution is mixed with neutral protease solution, the blend should be used immediately.

**Inactivation and inhibitors** The dissociation process can be reduced, e.g. by cooling down or diluting 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 AF-1 GMP Grade is not intended for direct application in humans. It is suitable for cell isolation from several tissue types intended for clinical application or transplantation into humans.