

## PRODUCT INFORMATION

### Neutral Protease AF GMP Grade

Cat. No. 30306

**Introduction** Neutral protease from *Clostridium histolyticum* is a proteolytic enzyme that can cleave peptide bonds in proteins such as collagen, proteoglycan, fibronectin, elastin, etc.

Due to these properties, it is suitable for the isolation of a broad range of cell types by tissue dissociation.

**Description** Neutral Protease AF GMP Grade is manufactured according to cGMP guidelines using a production process completely free of animal-based components. In this way the introduction of any potential animal-derived pathogen is excluded.

Neutral Protease AF GMP Grade is chromatographically purified and largely free from collagenolytic activity (PZ U) as well as endotoxins.

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

**Specification**

|                           |                    |
|---------------------------|--------------------|
| Neutral protease activity | ≥ 0.50 U/mg (DMC)  |
|                           | ≥ 100 U/vial (DMC) |
| TAMC                      | ≤ 10/vial          |
| TYMC                      | ≤ 10/vial          |
| Bacterial endotoxins      | ≤ 100.0 EU/mg      |

**Application** Neutral Protease AF GMP Grade is suitable for cell isolation from several tissue types for clinical applications.

It is often used in combination with Collagenase AF-1 GMP Grade (Cat. No. 17457).

**Storage conditions** Neutral Protease AF 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** For each lot a customer-specific certificate of analysis is provided.

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



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## Neutral Protease AF GMP Grade

### Instructions for use:

**General** Neutral Protease AF GMP Grade, often in combination with Collagenase AF-1 GMP Grade (Cat. No. 17457), is suitable for the dissociation of sensitive cells from several tissues, such as pancreas, liver and dental pulp. It has been shown to be highly effective for the isolation of islets of Langerhans from human pancreas intended for transplantation.

**Tissue dissociation** Recommended starting concentrations for isolation of islets of Langerhans from human pancreas:  
Collagenase AF-1 GMP Grade: 15 – 20 PZ U/g tissue  
Neutral Protease AF GMP Grade: 0.8 – 1.5 DMC U/g tissue  
Neutral Protease AF GMP Grade is provided in vials with  $\geq 100$  DMC U.  
The appropriate neutral protease concentration depends on the tissue type and origin as well as on the applied isolation procedure.  
If you have more questions concerning your application, you are welcome to contact the product management team at [crescent@creschem.com](mailto:crescent@creschem.com)

**Stock solution** A stock solution of Neutral Protease AF GMP Grade can be prepared by dissolving the enzyme in buffer or water. The enzyme solution should be constantly stored on ice.  
Since neutral protease depends on calcium, absolutely no calcium chelating agents (e.g. EDTA) should be present at all. If desired, a buffer with  $\geq 2$  mM  $\text{Ca}^{2+}$  can be used.  
Reconstituted Neutral Protease AF GMP Grade can be filtered, aliquoted and stored at  $-20$  °C. 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 neutral protease concentration.  
The working solution should be constantly stored on ice until use.  
If Neutral Protease AF GMP Grade solution is mixed with collagenase 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.  
Inhibitors of neutral protease include chelating agents like EDTA.

**Important note** Neutral Protease AF GMP Grade is not intended for direct application in humans.