

# TrypsiNNex<sup>®</sup>

Storage, handling and dilution guidelines



# TrypsiNNex<sup>®</sup>

## Storage, handling and dilution guidelines

### 1. Introduction

TrypsiNNex<sup>®</sup> is a high-quality recombinant trypsin produced according to cGMP guidelines<sup>1</sup> for a wide range of use in bioprocessing including cell dissociation, virus activation and protein modification. TrypsiNNex<sup>®</sup> is recombinantly expressed in *E. coli* with the trypsin gene sequence being identical to porcine trypsin. Being produced in dedicated production facilities that meet standards for non-animal origin, TrypsiNNex does not contain any animal-derived components.

Trypsin is a serine protease that hydrolyses peptide and protein substrates, specifically targeting the carboxyl side of arginine and lysine residues which are not followed by a proline. In addition to protein and peptide substrates, trypsin is prone to autolysis, where trypsin degrades itself. The  $\beta$ - and  $\alpha$ -trypsin content is a direct quality measure and is reported in the TrypsiNNex<sup>®</sup> Certificate of Analysis.

Trypsin activity is highly dependent on enzyme concentration, temperature, and pH. TrypsiNNex<sup>®</sup> is a highly concentrated product and, therefore, is formulated in a buffer that stabilizes the product in its most intact  $\beta$ -trypsin form. Proper handling of the TrypsiNNex<sup>®</sup> product will prolong the stability and content of  $\beta$ -trypsin and thus the high trypsin activity.

### 2. Storage of TrypsiNNex<sup>®</sup>

TrypsiNNex<sup>®</sup> is shipped on dry ice. Immediately upon receipt TrypsiNNex<sup>®</sup> should be stored at  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$  or lower to avoid any degradation and to ensure compliance with the specifications. Hence, under these conditions TrypsiNNex<sup>®</sup> is stable during its shelf-life period of 3 years<sup>2</sup> from the date of manufacture. Freeze/thawing cycles should be kept to a minimum and in accordance with thawing details listed below.

### 3. Thawing and handling temperatures for TrypsiNNex<sup>®</sup>

TrypsiNNex<sup>®</sup> must be thawed at temperatures between  $0^{\circ}\text{C} - 5^{\circ}\text{C}$  to avoid impact on enzyme activity. It is recommended to thaw TrypsiNNex<sup>®</sup> in a refrigerator at  $4^{\circ}\text{C}$ . Thawed TrypsiNNex should be handled at temperatures between  $0^{\circ}\text{C} - 5^{\circ}\text{C}$ . Handling at these temperatures should be kept at a minimum and not exceed 1.5 hours in total across the product life span. TrypsiNNex<sup>®</sup> exhibits good freeze-thaw stability. It is acceptable to store aliquots of undiluted TrypsiNNex<sup>®</sup> at  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$  or lower to avoid repeated freeze-thaw cycles.

1. TrypsiNNex<sup>®</sup> is GMP manufactured in accordance with IPEC's guideline "The Joint Good Manufacturing Practices Guide for Pharmaceutical excipients" (2017) and based on DS/EN ISO 9001.
2. Based on development studies.

## 4. Preparation and handling of TrypsiNNex<sup>®</sup> work solutions

### 4.1 TrypsiNNex<sup>®</sup> work solutions for cell dissociation

Cell dissociation studies using TrypsiNNex<sup>®</sup> have been conducted for various types of vaccine manufacturing cell lines as well as in mesenchymal stem cells. Based on these studies, the work range for TrypsiNNex<sup>®</sup> for use in cell dissociation is 30-1,000 Units/mL (USP trypsin units) depending on the cell type<sup>3</sup>. TrypsiNNex<sup>®</sup> would typically be diluted in phosphate-buffered saline (PBS) or Hanks' balanced salt solution (HBSS). The buffer solution may be supplemented with EDTA to increase the cell dissociation kinetics, if the further protocol allows the presence of EDTA. The typical EDTA buffer concentrations would be 0.5 - 1 mM EDTA. The work solution should be sterile filtered before use.

1. Prepare a sterile solution of PBS with 0.5 mM EDTA, and pre-cool to 4°C.
2. Calculate volume of TrypsiNNex<sup>®</sup> to reach work solution activity using Certificate of Analysis.
3. Add TrypsiNNex<sup>®</sup> directly to the PBS/EDTA solution, using a micropipette, followed by mixing, and sterile-filtering of the solution into pre-sterilized bottles or bags.

### 4.2 Storage of TrypsiNNex<sup>®</sup> work solutions

TrypsiNNex<sup>®</sup> work solutions at concentrations for cell dissociation or similar should be stored at max. 4°C. In general, degradation will occur in thawed TrypsiNNex solutions and the degradation will increase with temperature. Internal application studies indicate that 70 U/ml work solutions stored at 4°C may stay stable during the first 30 days of storage<sup>3</sup>. Additional information is available from the TrypsiNNex<sup>®</sup> "Statement regarding recommended storage conditions".

Based on general available information on the behavior of autolytic proteases, storage of TrypsiNNex<sup>®</sup> at intermediate concentrations (>10,000 Units/mL) at pH >2 should be avoided. If storage of TrypsiNNex<sup>®</sup> at intermediate concentrations is needed, it is recommended to maintain TrypsiNNex<sup>®</sup> in the formulation buffer (10 mM HCl, 20 mM CaCl<sub>2</sub>).

## 5. Safety

Please refer to the TrypsiNNex<sup>®</sup> Safety Data Sheet (SDS) at [novonordiskpharmatech.com](http://novonordiskpharmatech.com) for hazard information.

3. TrypsiNNex<sup>®</sup> Application Note – Cell Dissociation. Available upon request.

#### Please note:

The information contained herein is to our best knowledge true and accurate, but all recommendations or suggestions are made without guarantee since the conditions of use are beyond our control.

Novo Nordisk Pharmatech A/S disclaims any liability incurred with the use of these data or suggestions.

Novo Nordisk Pharmatech A/S  
Københavnsvej 216  
4600 Køge, Denmark

+45 5667 1000  
nnprinfo@novonordisk.com  
novonordiskpharmatech.com

**Novo Nordisk  
Pharmatech A/S**

