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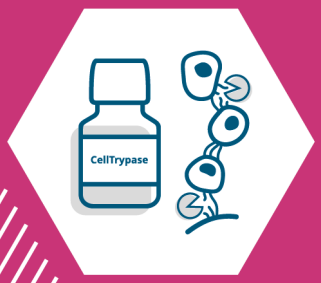

PRODUCT INFORMATION

CellTrypase

Recombinant *Fusarium oxysporum* trypsin-like enzyme, liquid.

CellTrypase is a recombinant, animal origin-free trypsin-like enzyme of fungal origin (*Fusarium oxysporum*), produced by microbial fermentation using *Bacillus* sp. It is intended for gentle and efficient dissociation of adherent cells in biopharmaceutical manufacturing and research applications. CellTrypase can be used as an alternative for trypsin proteases of different origin as well as other cell dissociation enzymes. Fully manufactured under GMP standards, CellTrypase offers high purity as well as gentle and reliable performance across a wide range of cell types.

1 Typical Applications

CellTrypase enables gentle and efficient dissociation of adherent and aggregating cells in biopharmaceutical processes. Manufactured without animal-derived components and in accordance with Good Manufacturing Practices (GMP), CellTrypase is well-suited for use in GMP-compliant production of therapeutic products. The enzyme performs reliably across various cell culture-based manufacturing and research applications. By maintaining cell viability and function, it supports robust and reproducible bioprocessing outcomes:

- Production of viral vectors for cell and gene therapies
- Manufacturing of viral vaccines
- Passaging and expansion of sensitive cell types such as iPSCs and hESCs
- Cell banking and differentiation workflows
- Isolation of primary cells
- Organoid culture and 3D cell models

CellTrypase is particularly suitable for workflows requiring high purity, reproducibility, and minimized risk of contamination.

2 Compliance

CellTrypase is intended for use in manufacturing processes of biopharmaceuticals such as viral vectors, vaccines, and cell-based therapies. The enzyme is available in two quality grades covering use in both early-stage development and GMP-compliant production processes. Both quality grades are manufactured without the use of antibiotics, animal-derived raw materials, or materials associated with TSE/BSE risk.

CellTrypase GMP-grade is manufactured and distributed under a certified Quality Management System in compliance with Good Manufacturing and Distribution Practice (GMP/GDP) according to the EXCiPACT® Certification Standard for pharmaceutical excipients. Comprehensive regulatory documentation for GMP-grade CellTrypase is available upon request to support customers with product filings and regulatory submissions.

Version 3.0, valid as of May. 12, 2026

In addition, a CellTrypase R&D-grade is available for research and process development purposes. This grade is produced under a Quality Management System according to ISO 9001 quality standards, with less stringent requirements regarding documentation, storage, and distribution. It is intended for early-stage development where fast and flexible access to raw materials is essential.

From a technical performance perspective, both quality grades are equivalent and meet the same analytical criteria of product specifications.

	GMP-grade CellTrypase	R&D-grade CellTrypase
Intended Use	Commercial manufacturing of biologics under GMP	Research, process development, preclinical stage
Quality standard	EXCiPACT® GMP/GDP for pharmaceutical excipients	DIN EN ISO 9001:2015
Manufacturing Environment	GMP-compliant production with full supply chain control. Manufactured with animal origin-free equipment.	Identical manufacturing process, without GMP documentation requirements.
Documentation	Full GMP documentation & traceability	Simplified documentation
Origin	Recombinant fungal trypsin-like enzyme (*Fusarium oxysporum*)	
Performance	Gentle and efficient cell dissociation preserving viability & function.	
Compatibility	Compatible with existing cell culture protocols. Can be used as a direct alternative to commonly used cell dissociation enzymes.	
Inactivation Method	No inactivation required. Dilution in buffer or medium is sufficient.	

3 Cell Dissociation & Handling Guidelines

General Use Instruction

CellTrypase can be used as an alternative for a wide range of commonly used cell dissociation enzymes, including fungal trypsin-like enzymes, recombinant porcine trypsin and other animal-derived alternatives.

For standard applications:

- **Pre-warm** CellTrypase and culture medium to room temperature or 37 °C. Warmer temperatures typically accelerate detachment.
- **Remove the spent medium** and rinse the cell monolayer with phosphate-buffered saline (PBS).
- **Add CellTrypase** to fully cover the cell layer (approx. 1 mL enzyme solution per 25 cm² surface area).
- **Incubate** until cells detach. Monitor visually and gently tap the cell culture vessel if needed.
  Optional: If cells do not fully detach, gently pipette up and down to aid dissociation. This can help ensure complete separation, especially for sensitive or tightly adherent cell types.
- **Quench enzymatic activity** by adding fresh medium. No trypsin inhibitor is required.
  Optional: For complete CellTrypase removal, centrifuge and resuspend cells in fresh medium.

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Dilution of 10x Stock Solution

CellTrypase is available as a ready-to-use 1x solution or as a 10x concentrated stock for dilution. The 10x format may be used undiluted for fast detachment of strongly adherent cells or diluted to 1x in PBS containing 1 mM EDTA. Dilution should be performed under sterile conditions. For most applications, a 1x working concentration is sufficient to ensure gentle and efficient cell release while maintaining high viability.

For detailed application data, stability information, and cell-specific performance metrics, please refer to the CellTrypase Application Note.

Performance Across Cell Lines

The table below summarizes CellTrypase performance in representative cell lines. Results demonstrate efficient detachment, high viability, and excellent recovery across diverse cell types:

Cell line	Growth Media	Release Time [mm:ss]	Mean Viability [% of total]	Yield [% of control]
CHO-K1	DMEM/F12 + 10% FBS + 1 mg/mL geneticin	02:25	98%	106%
HEK 293	DMEM + GlutaMax + 10% FBS + 2 mM glutamine	02:27	95%	100%
MDCK	EMEM + 10% FBS	23:32	99%	102%
Vero	EMEM + 10% FBS	04:07	99%	103%

Cell dissociation performance using CellTrypase (1x working solution). Control refers to an equivalent amount of the current industry standard trypsin-like enzyme.

4 Enzyme Purity

CellTrypase meets high purity standards. A representative chromatogram comparing CellTrypase and the current industry standard trypsin-like enzyme (both as 10x concentrated stock solutions) shows sharp overlapping peaks for the two products (**Figure 1**). However, the chromatogram of the industry standard displays a broader peak base with small additional signals. These may reflect the presence of impurities, inactive enzyme fragments or undesired proteolytic side products. The single, well-defined peak observed for CellTrypase indicates a highly purified enzyme preparation with minimal impurities or degradation products. High enzyme purity supports reproducible performance and specific activity, while minimizing the risk of non-specific proteolytic effects in sensitive bioprocessing applications.

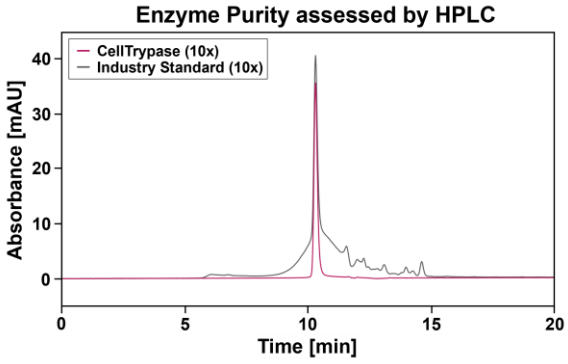

Figure 1 Purity profile of CellTrypase. The chromatographic profiles of 10x concentrated stock solutions of GMP-grade CellTrypase (pink) and the current industry standard trypsin-like enzyme (grey) were recorded using HPLC. It shows CellTrypase to be highly consistent and well-defined, with a distinct main peak and minimal by-products compared to the current industry standard.

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5 Inactivation & Removal of CellTrypase

Unlike porcine trypsin, CellTrypase does not require inactivation with trypsin inhibitors. Enzymatic activity can be reduced to a non-critical level for cells by simple dilution in buffer or medium. If needed, the enzyme can be removed from cell cultures by centrifugation, followed by removal of the enzyme-containing medium and resuspension of the cells in fresh medium.

6 Detection of Residual Enzyme

Residual CellTrypase can be detected and quantified using commercially available ELISA kits designed for recombinant trypsin-like enzymes of fungal origin. Compatibility with such assay formats has been experimentally verified, enabling integration into standard downstream analytics.

7 Storage Conditions & Stability

CellTrypase GMP-grade and R&D-grade demonstrate a confirmed stability of at least 18 months from the date of manufacture, based on available stability data, when stored at the recommended temperature of 2–8 °C.

Notes: Temporary exposure to room temperature or above (e.g., during shipment) does not impact enzyme activity. However, long-term storage above the recommended temperature range should be avoided. In particular, extended exposure to temperatures close to the enzyme's activity optimum (37 °C) will lead to a gradual loss of enzymatic activity due to the proteolytic nature of CellTrypase. The long-term stability of CellTrypase is currently under investigation.

8 Packaging Information

CellTrypase is filled into non-pyrogenic PET containers with HDPE screw caps. Each unit is secured with a tamper-evident shrink seal to ensure product integrity. All primary packaging materials comply with USP Class VI requirements for pharmaceutical use.
While CellTrypase should be stored at 2–8 °C, it can be shipped at ambient temperature without compromising product quality. This is supported by real-time stability data and transport stress testing.

9 Enzyme Characteristics

CellTrypase is a serine protease that specifically cleaves peptide bonds at the carboxyl side of lysine and arginine residues. This targeted activity enables controlled dissociation of attachment-dependent cells while preserving membrane integrity and cellular function. The enzyme's recombinant origin and defined substrate specificity result in:

- Consistent proteolytic performance across cell types.
- Reduced off-target cleavage, minimizing cellular stress.
- Gentle detachment, supporting downstream applications such as passaging, banking, or differentiation.

Molecular weight (calculated)	22 kDa[1]
pH optimum	pH 7.0 – 10.0
Temperature optimum	20 – 37 °C

[1] Calculated using Clone Manager 12 Professional Edition (Sci Ed Software); reflects calculation acc. to Lehninger

10 Product Specification

Recombinant trypsin-like enzyme of fungal origin (*Fusarium oxysporum*), produced by microbial fermentation using *Bacillus* sp. The production strain employed in the manufacturing of the product is a Genetically Modified Organism (GMO) of safety level S1.
The enzyme is supplied as liquid and formulated in PBS, 1.1 mM EDTA.

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To support a wide range of cell culture applications, CellTrypase is available in two concentration formats.

- Ready-to-use solution (1x concentration): Directly applicable without dilution.
- Concentrated stock solution (10x concentration): Can be diluted to 1x or used undiluted. Undiluted use of the 10x stock enables faster yet gentle dissociation of tightly adherent cells.

The GMP-grade product is manufactured under a certified Quality Management System in compliance with GMP according to the EXCiPACT® Certification Standard for pharmaceutical excipients. The R&D-grade variant is produced under a Quality Management System in compliance with ISO 9001 standards.

In order to ensure a constant and high-quality level for CellTrypase, each batch must fulfill the in-house acceptance criteria for the parameters listed below.

Parameter	Method	Specification
Appearance	Visual Inspection	Clear, transparent to slightly colored solution
Activity	Photometric[2]	(1x) 0.6 kU/L – 1.2 kU/L (10x) 6 kU/L – 12 kU/L
Identity	Determination by HPLC	Identity complies
Purity	Determination by HPLC	≥ 95 %
pH	Ph. Eur. 2.2.3	7.1 – 7.6
Osmolality	Ph. Eur. 2.2.35	270 – 320 mOsm/kg
Endotoxin level	LAL-Test Ph. Eur. 2.6.14/USP <85>, Method D	(1x) ≤ 1 EU/mL (10x) ≤ 10 EU/mL
Mycoplasmas	qPCR Detection Assay acc. to Ph. Eur. 2.6.7	Negative
Sterility testing	Ph. Eur. 2.6.1/USP <71>	Pass

[2] Unit-Definition: One unit (U) corresponds to the amount of CellTrypase that forms 1 µmol *p*-Nitroaniline per minute at 37 °C from 8 mM L-AAPA at pH 8.0.

11 Sales & Contact

GMP Products for biopharmaceutical manufacturing

Product	Art. No	Conc.	Size	
CellTrypase (1x), 100 mL, GMP	22103-1X-100-G	1x	100 mL	Produced under EXCiPACT® GMP standard
CellTrypase (10x), 100 mL, GMP	22103-10X-100-G	10x	100 mL	Produced under EXCiPACT® GMP standard
CellTrypase (1x), 500 mL, GMP	22103-1X-500-G	1x	500 mL	Produced under EXCiPACT® GMP standard
CellTrypase (10x), 500 mL, GMP	22103-10X-500-G	10x	500 mL	Produced under EXCiPACT® GMP standard

Products for use in research and development

Product	Art. No	Conc.	Size	
CellTrypase (1x), 100 mL	22103-1X-100	1x	100 mL	Produced under ISO 9001 standard
CellTrypase (10x), 100 mL	22103-10X-100	10x	100 mL	Produced under ISO 9001 standard
CellTrypase (1x), 500 mL	22103-1X-500	1x	500 mL	Produced under ISO 9001 standard
CellTrypase (10x), 500 mL	22103-10X-500	10x	500 mL	Produced under ISO 9001 standard

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