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Technical Brief

CellPrime® rTrypsin Recombinant trypsin

Stem cell propagation protocols and vaccine manufacturing processes in adherent cells, as well as precursor maturation in therapeutic recombinant insulin production, have relied on pancreatic trypsin as a dissociation agent and manufacturing aid respectively. Pancreatic trypsin is a ~23 kD serine protease that specifically cleaves the peptide backbone after arginine or lysine residues.

Native pancreatic trypsin, however, has a principal drawback in that it is an ill-defined mixture of β- and α-trypsin, laced with varying amounts of chymotrypsin depending on purity. Furthermore, animal origin (AO) native trypsin poses a significant risk with regard to introducing adventitious viral agents into the biomanufacturing process. Well-publicized incidents of this nature have led to plant shutdowns and disruptions of therapeutic drug supply to patients.

This precedent ushered in a tectonic shift of biopharmaceutical quality paradigms, with governmental agencies like FDA, EMEA, WHO and industry associations increasingly advocating the replacement of animal origin process components with recombinant and non-animal origin (NAO) materials whenever possible, to improve the biopharmaceutical safety record.

In order to meet growing market needs, we are now adding a proprietary recombinant trypsin to our CellPrime® portfolio of non-animal origin recombinant cell culture supplements and manufacturing aids.

Product description

CellPrime® rTrypsin is recombinantly expressed by a synthetic DNA construct that encodes the porcine gene sequence in the yeast Pichia pastoris. It is manufactured in accordance with good manufacturing practice (GMP) guidelines in a dedicated state-of-the-art production facility that meets non-animal origin requirements.

As such, CellPrime® rTrypsin does not contain components of animal origin in either the

- Master Cell Bank (MCB)
- Working Cell Bank (WCB)
- raw materials for production
- manufacturing process, or
- the final product.

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Biochemical characterization of CellPrime® rTrypsin

CellPrime® rTrypsin is initially manufactured as an inactive r-trypsinogen precursor. This precursor molecule is subsequently activated under carefully controlled conditions to yield, by auto-catalytic conversion, a reproducibly balanced mixture of α- and β-trypsin moieties (β-trypsin >70 %, α–trypsin < 20 %) with only minor quantities of extraneous material, most likely autolytic degradation intermediates.

The RP-HPLC profile of CellPrime® rTrypsin shown (Fig. 1) meets the acceptance criteria for purity as described in USP 38 (Chapter 89, Enzymes Used as Ancillary Materials in Pharmaceutical Manufacturing) using a USP r-trypsin reference standard.

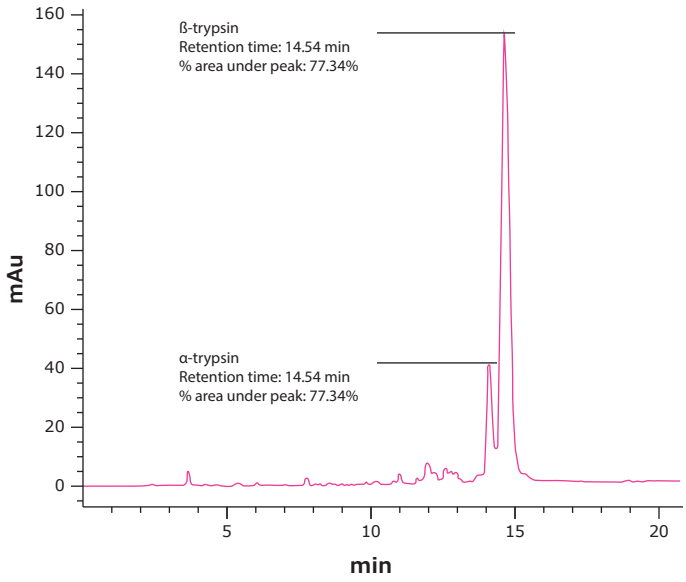

Figure 1

RP-HPLC chromatogram resolving the α- and β- subunits of CellPrime® rTrypsin. Peak area indicates 77 % β-trypsin and 14 % α-trypsin. The profile highlights the high degree of CellPrime® rTrypsin purity and the absence of degradation products or other impurities.

As would be expected for a highly purified recombinant enzyme preparation, CellPrime® rTrypsin exhibits a consistent and high specific activity (expressed as activity units measured using carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate substrate – Chromozym TRY – as per the USP 38 monograph release assay).

Previously, r-trypsin activity had been expressed as USP activity units measured under the conditions described in an older (USP 32) monograph for the characterization of crystallized porcine trypsin using N-benzoyl-L-arginine ethyl ester hydrochloride (BAEE) as the substrate. One unit of trypsin activity using carbobenzoxy-valyl-glycyl-arginine-4-nitril-anilide acetate (Chromozym TRY) as the substrate corresponds to 21 USP trypsin (BAEE) units. This conversion factor has been used in Table 1 below, which shows the specific activity measured for several preparations of CellPrime® rTrypsin in liquid and powder grades. It is the conversion factor defined in the recent 2015 monograph (USP 38 [89]) for r-trypsin using the USP r-trypsin reference standard.

Table 1: CellPrime® rTrypsin, specific activity measurements using new and old USP monograph assays.

	Specific activity (Chromozym TRY assay units), U/mg protein	Specific activity (BAEE, USP assay units), U/mg protein
CellPrime® rTrypsin, liquid		
Lot 1	244	5,128
Lot 2	244	5,116
Lot 3	247	5,176
CellPrime® rTrypsin, powder		
Lot 1	231	4,844
Lot 2	244	5,121
Lot 3	245	5,146

Applications for recombinant trypsin

Recombinant trypsin is most frequently used by customers in the following applications.

- Vaccine production: to dissociate and resuspend adherent cell lines during seed train extension and harvest
- General life science R&D cell culturing: to passage adherent cells
- Adult human stem cell culture: to dislodge adherent cells grown on carriers (e.g., hMSC) while preserving the structural and functional integrity of cell surface markers
- Recombinant insulin manufacturing: as protease in the maturation of proinsulin to active insulin

Cell dissociation studies using CellPrime® rTrypsin on cell lines commonly used in vaccine manufacturing

Trypsinization of adherent cell lines is a standard practice used in general life science R&D and in virus-borne vaccine manufacturing processes employing cell lines such as MRC-5 (Human Fetal Lung Fibroblast Cells), MDCK (Madin-Darby Canine Kidney Cells), and Vero Cells (African Green Monkey Kidney Epithelial Cells).

Commercially available ready-to-use porcine trypsin/trypsin-EDTA (1x) solutions typically contain the enzyme at concentrations ranging from 0.025 to 0.5 % (w/v), based on fluctuating potency of the trypsin activity. Researchers need to establish, for every cell line and for every enzyme lot ordered, the optimal time needed to afford gentle dissociation of cells while minimizing detrimental side effects. Incubating cells with too high a trypsin concentration or for too long a time can lead to irreversible membrane or cell surface marker damage and thus loss of function followed by cell death.

For the cell dissociation study, CellPrime® rTrypsin in liquid and powder (lyophilisate) grade was tested on the vaccine manufacturing cell lines above, which included both weakly and strongly adherent cells.

Cell viability was ascertained using a standard trypan blue exclusion assay and, after overnight recovery, by visual inspection of the cells for morphological abnormalities under a light microscope.

Cells were seeded at 0.7 x 106 cells/T 25 flask, incubated for 48 hrs (37 °C, 5 % CO2) until approximately 90 % confluency was reached, washed with PBS (1x), and incubated in duplicate assays for 3 passages.

Dissociation times were recorded by cell line and the CellPrime® rTrypsin preparation used. CellPrime® rTrypsin liquid concentration is represented as weight of protein over volume of assay buffer (w/v). CellPrime® rTrypsin powder concentrations refer to the weight of powder over volume of assay buffer (w/v) where 1 g of CellPrime® rTrypsin powder consistently contains > 0.6 g of protein. For both liquid and powder, CellPrime® rTrypsin concentrations of 0.05, 0.1, and 0.25 % (w/v) were benchmarked against commercially available porcine trypsin (GIBCO® Trypsin from Thermo Fisher). The results are summarized in Table 2.

Table 2: Cell dissociation with CellPrime® rTrypsin.

Cell line	Cell viability	Concentration	Dissociation time (minutes)	
			CellPrime® rTrypsin, liquid	CellPrime® rTrypsin, powder
MRC-5	> 95 %	0.05 % (w/v)	3.15 ± 0.03	3.25 ± 0.04
Vero	> 95 %	0.1 % (w/v)	5.61 ± 0.2	5.67 ± 0.04
MDCK	> 95 %	0.1 % (w/v)	5.68 ± 0.16	5.69 ± 0.02

At any given concentration, on the same cell line and in this assay, CellPrime® rTrypsin liquid and powder yielded comparable results in dislocating adherent cells while maintaining cell morphology and viability.

Table 2 shows the optimal concentrations determined for the respective cell lines. Increasing the concentrations shown for Vero and MDCK 2.5-fold did not appreciably shorten the observed dissociation times. A shortening of the dissociation time was observed for MRC-5 cells upon doubling of the enzyme concentration shown above. However, an associated increase in the percentage of morphologically abnormal cells was observed, and thus this concentration should be avoided.

In conclusion, and as a guideline for cell lines other than those described above, weakly adherent cells are optimally dissociated by CellPrime® rTrypsin powder and liquid at 0.05 % (w/v), whereas strongly adherent cells require concentrations of 0.1 % (w/v) of the product.

Separate studies have successfully been carried out with human mesenchymal stem cells (hMSCs) grown on solid supports in a 3D-bioreactor format. The results of this investigation are reported in a separate data sheet.

Use of CellPrime® rTrypsin in recombinant insulin manufacturing

Regulatory authorities have mandated that animal origin (AO) manufacturing aids such as porcine trypsin be replaced with recombinant and entirely non-animal origin (NAO) equivalents whenever feasible, to enhance the virus safety of the manufactured biologics.

Porcine trypsin is still widely used in the production of recombinant insulin, where it serves to convert recombinantly expressed precursor molecules into the mature, active insulin molecule (Fig 2). CellPrime® rTrypsin is a suitable and reliable replacement for porcine trypsin in these processes that can help eliminate the animal-sourced enzyme from the production templates of this widely prescribed biologic.

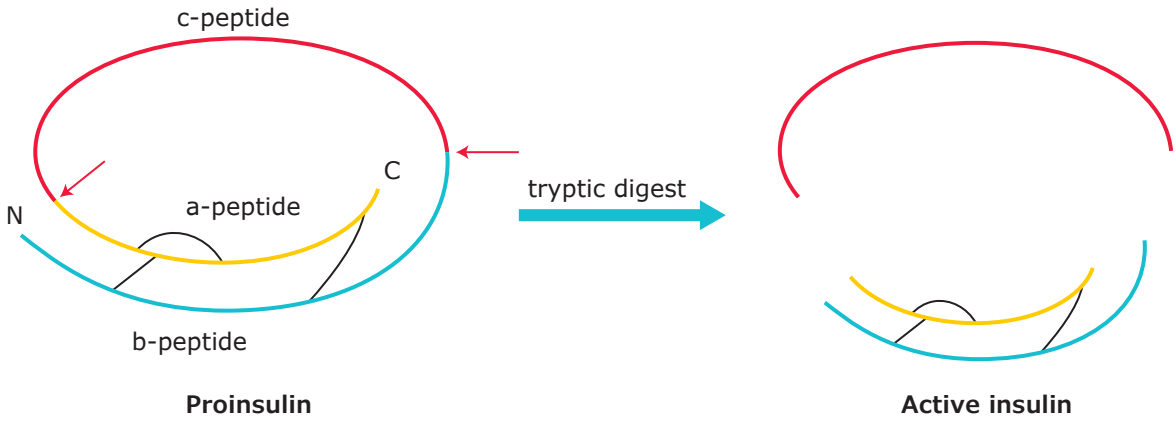

Figure 2

Conversion of proinsulin to insulin.

Proinsulin precursor molecules cleaved with trypsin to remove the c-peptide (connecting peptide) yield the active insulin.

To demonstrate the conversion of a proinsulin intermediate by CellPrime® rTrypsin, RP HPLC analyses were run on samples before and after incubation with suitable amounts of the enzyme.

Fig. 3a shows the production intermediate resolved by RP HPLC. The major peak eluting at 49 minutes is the proinsulin.

Fig. 3b shows an aliquot of the same starting material after cleavage by CellPrime® rTrypsin. The moiety eluting at 11 minutes represents mature insulin, whereas the material eluting at 28 minutes represents a process by-product that is subsequently removed from the mature insulin. (Data kindly provided by a manufacturer of recombinant therapeutic human insulin.)

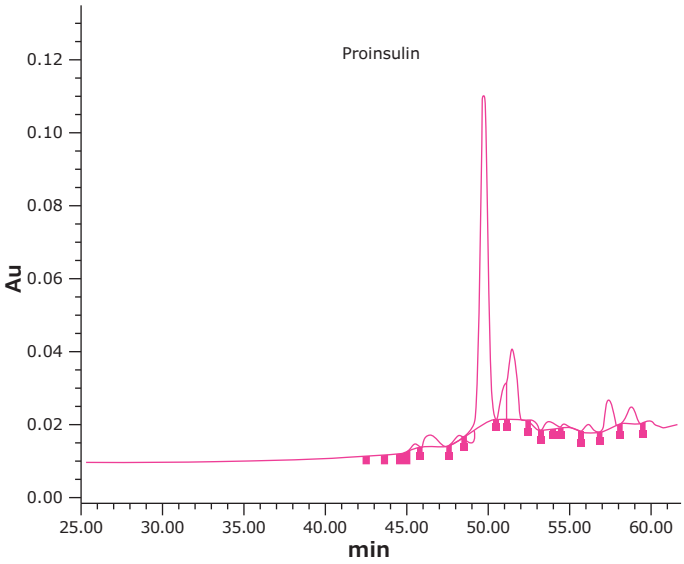

Figure 3a

Proinsulin resolved by RP-HPLC before cleavage with CellPrime® rTrypsin.

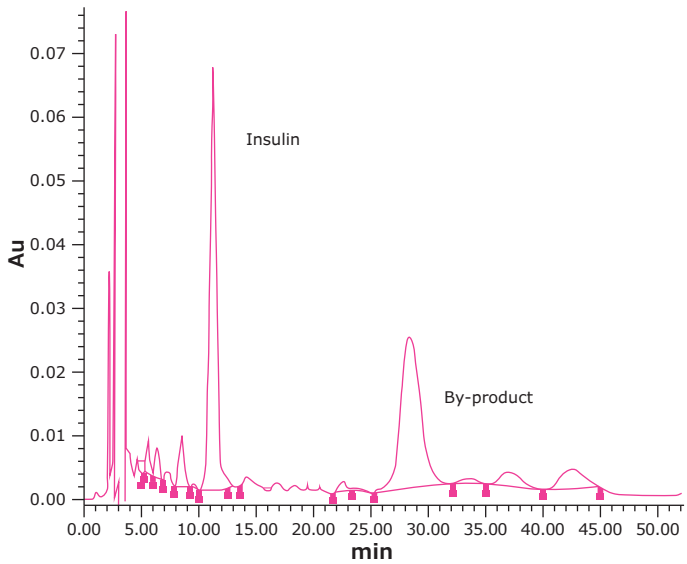

Figure 3b

Matured insulin resolved by RP-HPLC after cleavage with CellPrime® rTrypsin.

Taken together, this data demonstrates that CellPrime® rTrypsin is a suitable manufacturing aid that enables the production of recombinant insulin.

In conclusion, CellPrime® rTrypsin

- fulfills the quality criteria (purity and enzyme activity) set forth by USP 38 (89) for enzymes used as ancillary materials in (bio)pharmaceutical manufacturing processes (USP-compliant)
- complies with the highest market standards for non-animal origin (NAO) materials
- thereby increases process reliability and facilitates regulatory compliance
- is available as a liquid and powder stock with identical enzyme activity to satisfy different user requirements, and
- can be formulated as a sterile, GMP-manufactured solution to meet a variety of customer application needs.

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