Data Sheet

CellPrime® rTrypsin Recombinant Trypsin for Stem Cell Manufacturing

The long-term commercial viability of regenerative medicine therapies forecasts an increased need for high-quality non-animal origin materials to ensure a safe and sustainable supply. Trypsin has been recognized as a pivotal dissociation agent to harvest expanded stem cells for clinical use. Stem cell propagation protocols have relied on pancreatic trypsin as a dissociation agent. However, animal-origin native trypsin poses a significant risk with regard to introducing adventitious viral agents into the stem cell manufacturing process. In order to meet increased market needs for non-animal origin cell culture reagents, we have added a proprietary recombinant trypsin alternative to the CellPrime® portfolio.

CellPrime® rTrypsin helps you optimize and secure your stem cell manufacturing process. This non-animal origin, recombinant cell dissociation reagent is expressed by a synthetic DNA construct encoding the porcine gene sequence in the yeast *Pichia pastoris*.

CellPrime® rTrypsin is manufactured according to cGMP in a dedicated state-of-the-art production facility that meets non-animal origin requirements. Its activity is specified according to the USP <89> monograph for recombinant trypsin using the USP recombinant trypsin reference standard.

Benefits

- In line with the highest market standard for non-animal origin materials.
- Confirmed human stem cell harvest and performance profiles.
- High purity and consistency from batch to batch.
- Available in liquid stock solution or as bulk dry powder material.

EMD Millipore Corp. is a subsidiary of Merck KGaA, Darmstadt, Germany

Like all members of the recombinant CellPrime® portfolio, CellPrime® rTrypsin is a non-animal origin product that does not contain components of animal origin in the:

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

Applications beyond cell culture

Customers can also use recombinant trypsin in these applications:

- Vaccine production: as a processing enzyme or to dissociate and resuspend adherent cells producing viral particles or antigens.
- Therapeutic recombinant insulin manufacturing: as a protease in the maturation of proinsulin to active insulin.

Proven performance

CellPrime® rTrypsin is one of our many cell culture products that provides excellent quality, performance, and lot-to-lot consistency. In order to demonstrate these features, we performed a cell culture case study adressing the harvest of stem cells from flat culture systems or bioreactors.

Materials and methods

For the case study we used proprietary bone marrow-derived mesenchymal stem cells expanded in traditional lab-scale planar culture system as well as in a Mobius® bioreactor.

Serial passaging study in planar culture system
Bone marrow-derived human mesenchymal stem cells (MSCs) from a master cell bank were thawed and seeded in T25 flasks at 3000 cells/cm². Cells were expanded in FBS-supplemented DMEM medium for 4 successive passages over a period of 14 days at 37 °C in a 5 % CO_2 incubator. Four different lots of CellPrime® rTrypsin were used to detach cells for subsequent passaging and final harvest. Cell count and cell viability were measured at each passage using the Cellometer® Auto T4 Cell Counter (Nexcelom Bioscience, MA, USA). All conditions were run in duplicate.

Large-scale expansion and harvest in 50 L bioreactor
MSCs were grown on microcarriers in a Mobius® 50 L Single-use Bioreactor for 10 days using a modified fed-batch seed and feed strategy in human platelet lysate-supplemented αMEM. Total cell count was directly assessed on a daily basis from microcarrier samples using the Nucleocounter® NC-100™ device (ChemoMetec A/S, Denmark). On the harvest day, the microcarriers with attached cells were allowed to settle, and this material was washed with phosphate-buffered saline. CellPrime® rTrypsin was added and incubated at 37 °C with agitation for 15 minutes. Following neutralization with fresh medium, all the materials were passed through a normal flow filtration device to retain the microcarriers. Cell viability was checked following microcarrier separation and cell surface markers were analyzed using the Guava® easyCyte™ Flow Cytometry System.

Results

Serial passaging study in planar culture system
The objective was to evaluate the performance of different lots of CellPrime® rTrypsin in MSC applications over several passages. The study demonstrates that multiple lots of CellPrime® rTrypsin yielded the same population doublings over several passages (Figure 1).

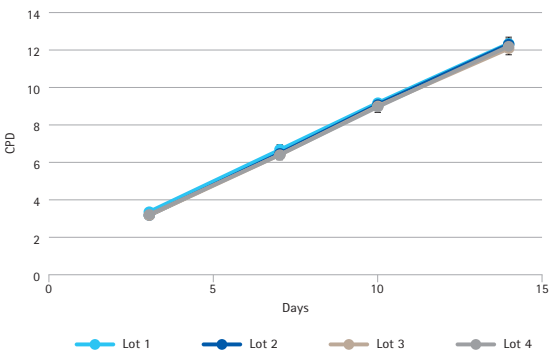

Figure 1
Cumulative population doublings (CPD) of MSCs expanded in 10 % FBS-supplemented DMEM medium in T25 flasks. Four different lots of CellPrime® rTrypsin solutions at 24 USP units/mL were used to detach cells for 4 subsequent passages.

These experiments also show the preservation of high cell viability when MSCs are detached from the cell culture surface and harvested with CellPrime® rTrypsin over multiple passages (Figure 2).

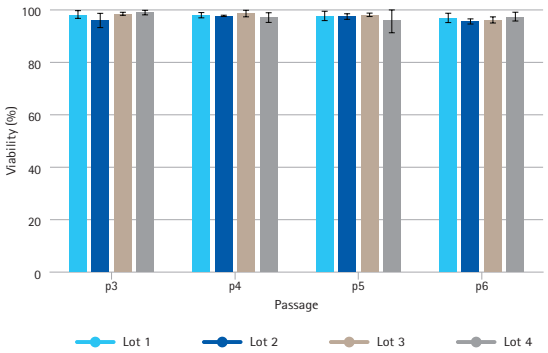

Figure 2

Cell viability measured after detaching MSCs from T25 flasks with CellPrime® rTrypsin for 4 consecutive passages.

Large-scale expansion and harvest in 50 L bioreactor

The objective was to confirm the performance of CellPrime® rTrypsin for harvesting MSCs attached and expanded onto microcarriers in the Mobius® 50 L bioreactor. Cells were cultivated for 15 days in αMEM supplemented with human platelet lysate (Figure 3).

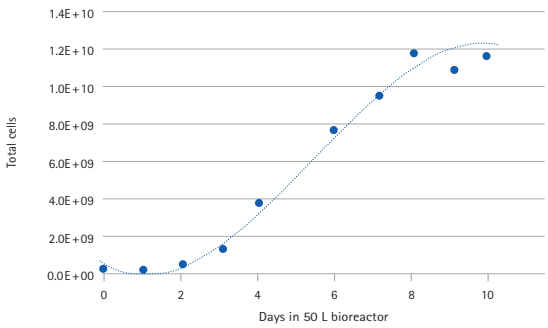

Figure 3

Daily total cell counts for MSCs expanded in Mobius® 50 L bioreactors. On harvest day 10, cells were detached from the microcarriers using CellPrime® rTrypsin (71.5 USP units per million cells).

At the point of harvest, all cells were detached from the microcarriers using CellPrime® rTrypsin and counted. A final harvest yield of 10.5 billion cells was achieved, reflecting a 35-fold increase in cell density through the use of this large-scale process, and cell viability following microcarrier separation remained high at 96 % (Figure 4A). Flow cytometry for a collection of positive and negative markers was used to assess MSC identity (Figure 4B).

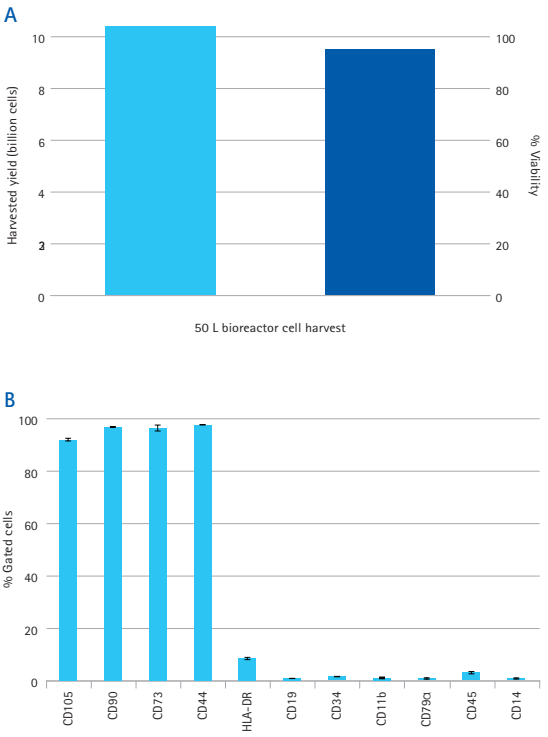

Figure 4

Harvest yield and cell viability (A) and FACS analysis of MSC markers (B) following microcarrier separation, after expansion in Mobius® 50 L bioreactors.

Conclusion

The studies described above demonstrate that CellPrime® rTrypsin can be used as a dissociation agent to harvest expanded stem cells, both in planar and bioreactor culture systems.

For the serial passaging study, the 4 different lots of CellPrime® rTrypsin reached comparable cell growth and maximum cell viability over multiple cell passages. For the large-scale study performed in a Mobius® 50 L bioreactor, excellent cell harvest yields without compromising stem cell quality were confirmed when using CellPrime® rTrypsin to harvest MSCs from microcarriers.

CellPrime® rTrypsin performs consistently across multiple lots and multiple culture systems with human mesenchymal stem cells. The current experiments confirm that CellPrime® rTrypsin works reliably for its intended application of promoting efficient harvest in stem cell manufacturing.

Storage and handling

- Store CellPrime® rTrypsin powder **refrigerated** at **2–8 °C** and keep on ice during handling. Weigh and dissolve lyophilisate into the buffer being used (e.g. 1 x PBS pH 7-7.4 w/o Ca^{2+} and Mg^{2+}).
- Store CellPrime® rTrypsin liquids **frozen** at -20 °C, aliquot (if necessary) upon receipt and only refreeze **once.** Discard the excess from the aliquots after diluting into the buffer being used for your application.

Usage

- For further manufacturing use only.
- For cell dissociation reagent use only.
- Not for human or therapeutic use.

Ordering information

CellPrime® rTrypsin is available in various pack sizes, both as lyophilized powder and as a liquid formulation.

Catalog number	Product name	Mega units	Pkg. size
1.06301.0001	CellPrime® rTrypsin recombinant Trypsin (powder)	0.119 MU	1 g
1.06301.0010	CellPrime® rTrypsin recombinant Trypsin (powder)	1.19 MU	10 g
1.06301.0050	CellPrime® rTrypsin recombinant Trypsin (powder)	5.95 MU	50 g
1.06302.0002	CellPrime® rTrypsin recombinant Trypsin (liquid)	0.013 MU	1 mL
1.06302.0010	CellPrime® rTrypsin recombinant Trypsin (liquid)	0.13 MU	10 mL
1.06302.0050	CellPrime® rTrypsin recombinant Trypsin (liquid)	0.65 MU	50 mL
1.06302.0200	CellPrime® rTrypsin recombinant Trypsin (liquid)	2.6 MU	200 mL

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