Data Sheet

CellPrime® r Insulin

Recombinant human insulin for improved cell culture performance

Mammalian cell culture biomanufacturing critically depends on selecting and blending high quality raw materials and supplements for upstream media formulations. Insulin has been recognized as a pivotal component for cellular carbohydrate metabolism that enhances cell growth and protein production. Due to its ability to extend the long-term viability of various cell lines, insulin is used in mammalian cell culture geared towards the production of monoclonal antibody therapeutics (mAbs), viral vaccines and a wide spectrum of other recombinant biomolecules (e.g., coagulation factors).

To optimize your cell culture media without sacrificing quality, EMD Millipore now offers CellPrime® r Insulin, a non-animal origin, recombinant supplement. CellPrime® r Insulin is produced by microbial expression as a recombinant human insulin precursor in *E.coli* (K12), followed by enzymatic cleavage and subsequent purification steps that yield a crystalline powder product.

Manufactured according to cGMP in a state-of-the-art, dedicated production facility that meets non-animal origin requirements, CellPrime® r Insulin complies with compendial standards defined in USP and EP monographs and is analyzed according to protocols defined therein.

Benefits

- In line with highest market standard for non-animal origin materials
- Traceable raw materials of non-animal origin
- Long-term viability of various cell lines
- Confirmed cell culture growth and performance profiles

Is your process free of animal-origin materials?

CellPrime® r Insulin meets EMD Millipore's stringent definition of non-animal origin for recombinant cell culture supplements , with no animal-derived components in any of the:

- Master Cell Bank (MCB)
- Working Cell Bank (WCB)
- Raw materials
- Manufacturing process
- Final product

Excellent performance

CellPrime® r Insulin is one of many EMD Millipore cell culture products that provide excellent quality, performance, and lot-to-lot consistency. In order to demonstrate these features, we performed a cell culture case study using a proprietary, insulin-dependent CHO cell line expressing a monoclonal antibody.

Materials and methods

Preparation of insulin stock solutions

Stock solutions of three lots of CellPrime® r Insulin and samples of commercially available recombinant insulin (to be referred to as insulin throughout this document) produced in yeast or *E.coli*, respectively, were made by dissolving the insulin crystals in hydrochloric acid (10 mM, pH 2-3). Unused material was refrozen and stored at -20 °C. Preliminary studies were conducted in order to ascertain that none of the preparations had a toxic effect on cell growth.

Serial passaging study

The selected insulin dependent CHO-DG44 cells were washed twice in our production medium lacking insulin. The cells were then resuspended (5 x 10^5 viable cells/mL) in 40 mL of production medium containing 4mg/L insulin to be tested, Puromycin and L-Glutamine in shake flasks. All conditions were run in triplicate.

For subsequent passaging, appropriate amounts of cells were pelleted and resuspended in fresh medium (containing insulin, Puromycin and L-Glutamine). After five passages, a termination batch was run to measure cellular performance (maximum cell density, viability and productivity). Cells were counted daily using a Vi-Cell® Cell Viability Analyzer and titer was measured using an Octet® QK instrument.

Assay for half minimal effective concentration (EC_{50})

The selected insulin dependent CHO-DG44 cells were washed twice in our production medium lacking insulin. The cells were resuspended (5 x 10^5 viable cells/mL) in 3.5 mL of production media containing no insulin, then cultured in 24 deep-well plates for a starvation period of 24 hours. After 24 hours, various insulin amounts ranging from 0 mg/L to 10 mg/L were added to the cell culture wells. Cells were counted daily using a Guava® easyCyte 5HT and titer was measured using an Octet® QK instrument.

Results

Serial passaging

The objective was to compare CellPrime® r Insulin against two different commercially available recombinant insulin products (expressed in yeast or *E.coli*). The insulin dependency of the cell line was confirmed in the first passage by the high doubling time of cells cultured in insulin-depleted medium and the subsequent death of these cells in the second passage (Figure 1). Cellular growth was monitored over an additional four passages. The study demonstrates that CellPrime® r Insulin consistently yields results similar to the two commercially available insulins.

Similar growth patterns, viability curves and titer profiles between each condition have been observed in a termination batch conducted at passage 5 of the serial passaging study, as shown in Figure 2 and 3.

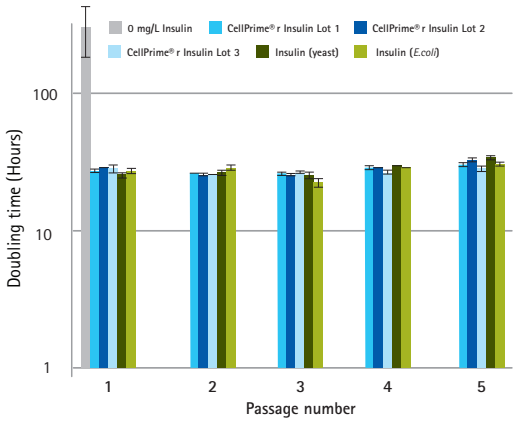

Figure 1.
Comparison of doubling times of CHO-DG44 cells in non-insulin containing basal media supplemented with 4 mg/L insulin from three different lots of CellPrime® r Insulin versus two commercially available insulin samples.

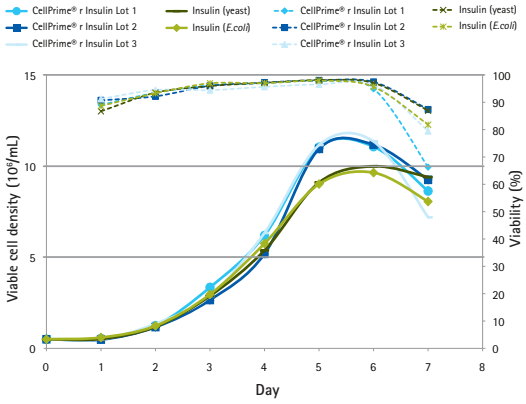

Figure 2.
Cell viability and viable cell density measured while culturing cells on three different lots of CellPrime® r Insulin in comparison to commercially available recombinant insulin. A termination batch was initiated after passage 5 of the serial passaging study (Figure 1).

EC_{50} study

The objective was to compare CellPrime® r Insulin against two different commercially available recombinant insulin products (expressed in yeast or *E.coli*) for the determination of the half-minimal effective concentration 50 (EC_{50}). The study demonstrates that CellPrime® r Insulin on day 5 consistently is on par with yeast insulin whereas more *E.coli* insulin is required to reach the maximum growth (Figure 4).

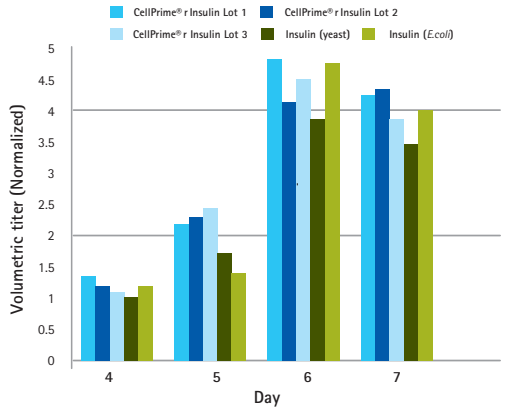

Figure 3.
Titer normalized to yeast sample on day 4 of culture. Three different lots of CellPrime® r Insulin in comparison to commercially available recombinant insulin are shown in a termination batch conducted at passage 5 of the serial passaging study.

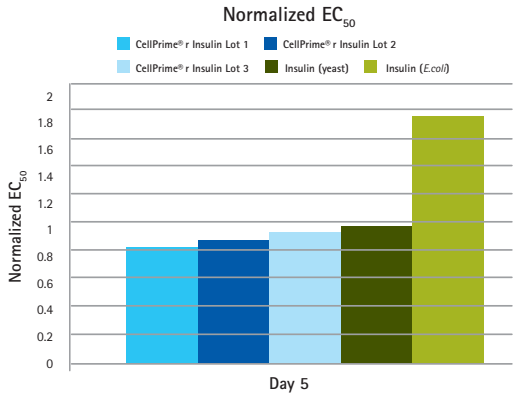

Figure 4.
Normalized EC_{50} benchmarked to yeast insulin

Conclusion

The studies described demonstrate that CellPrime® r Insulin consistently yields results comparable with or superior to two commercial insulins (expressed in yeast or *E.coli*) through serial passaging and EC_{50} experiments. For the serial passaging study, all conditions reached comparable maximal viable cell densities and the titer was not statistically different from one another. For the EC_{50} study, CellPrime® r Insulin lots and yeast insulin were on par with regard to potency whereas more *E.coli* insulin was needed to obtain the same effect.

The current experiments confirm that CellPrime® r Insulin works reliably for its intended application of promoting cellular growth in mammalian cell culture biomanufacturing.

Ordering information

Catalog number	Pkg. size
4512.01	1 g
4512.10	10 g
4512.50	50 g
4512.100	100 g
4512.1K	1.000 g

Storage

The product must be stored at -20 °C ± 2 °C and protected from light to avoid any potential degradation and to ensure compliance with the specification for the entire shelf life period.

Usage

- For further manufacturing use only.
- Not for human or therapeutic use.
- For cell culture media supplement use only.

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