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Pro293™ Serum-free and Suspension Adaptation for 293 Cells

Instructions for use

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I. Introduction

In the process of serum-free adaptation, it is important to monitor viability (>70%), cell growth and viral production for each medium formulation change. Pro293™a-CDM™ Medium, is for adherent cells, while Pro293™s-CDM™ Medium should be used for cells in suspension. Suggestions for adaptation of cells to serum-free Pro293™ Media are as follows.

For answers to frequently asked questions and citations regarding these products, please visit our knowledge center: **https://knowledge.lonza.com**

II. Cell expansion and serum reduction protocol

1. Expand 293 cells in control medium (classic culture medium, such as DMEM-F:12) supplemented with 10% FBS until 10-12 T-175 cm^2 flasks are confluent.

2. At confluence, perform a 1:2 split on half of the 10% FBS T-flasks (5-6 flasks) into 10-12 T-175 flasks containing control medium plus 2% FBS. Maintain these flasks in control medium plus 2% FBS with three control medium exchanges per week. If cells are detaching, centrifuge, and return cell pellet to the flask.

3. Cells in control medium plus 2% FBS should be maintained throughout the adaptation process as a backup.

4. Freeze cells at high cell density (about 2×10^7 cells/ml) in freezing medium consisting of control medium plus 2% FBS and 7.5% DMSO. Use the remaining flasks for serum-free adaptation for adherent and suspension culture.

III. Adherent culture: serum depletion protocol

1. Perform a 1:2 split on half of the confluent control flasks (5-6 flasks) into 10-12 T-175 flasks with Pro293™a-CDM™ Medium plus 2% FBS.

2. Maintain cells in 2.0% serum Pro293™ a-CDM™ Medium with three medium exchanges per week.

3. Reduce serum to 1.0% and maintain cells with three medium exchanges per week. Split 1:2 when confluent.

4. Reduce serum to 0.5% and maintain cells with three medium exchanges per week. Split 1:2 when confluent.

5. Reduce serum to 0.2% and maintain cells with three medium exchanges per week.

Split 1:2 when confluent into 100% Pro293™a-CDM™ Medium.

NOTE:

- This medium must be supplemented with 2-4 mM L-glutamine
- Use 60 mL medium for each T-175 flask
  If cells are detaching, centrifuge and return cell pellet to the flask
- Freeze six ampoules of cells (1-2 x 10^7 cells/mL) for each serum reduction
- Change medium on Monday, Wednesday and Friday
- Mix cells and remove mixed cell suspension for cell counting and viability
- Do not decrease serum concentration unless cells are growing, viable and attached. At lower serum concentrations, several passages at one serum level may be needed before further reductions are tolerated

IV. Suspension culture: serum depletion and suspension adaptation protocol

1. Seed spinners at 3-5 x 10^5 cells/mL with cells from an adherent culture previously growing in control medium supplemented with 10%, 5% or 2% FBS. Reduce the FBS supplementation in the Pro293™s-CDM™ Medium to the next successive level according to the schedule below.

2. Use 500 mL spinners with a working volume of 200 mL.

3. Over multiple passages, decrease FBS supplementation of Pro293™s-CDM™ Medium from 5% to 2%, to 1%, to 0.5%, to 0.2% to 0%.
   - Cell density should be at least 1.2 x 10^6 cells/mL prior to splitting. Fully adapted cells should routinely grow to 2-3 x 10^6 cells/mL
   - Split cells using no more than one part old medium with cells to three parts new medium
   - Each serum level will require at least two passages
   - The decision to reduce to the next level should be based on growth and viability at each passage

4. Maintain agitation with good mixing. Agitation is typically between 70-90 rpm.
   - Initially there may be cell clumping, but this will decrease with further reductions in FBS level, and will disappear in serum-free medium (100% Pro293™s-CDM™ Medium).

5. Maintain cells in serum-free Pro293™s-CDM™ Medium.

6. Count cells and check viability on a daily basis.
   - Maintain cell viability above 70%
   - If cell viability is below 70%, slow down the serum removal process
   - To enhance viability, feed 50 mL fresh medium daily, not to exceed 350 mL volume in 500 mL spinners

7. Inoculate new spinners based on viable cell number.

8. If cells are growing slowly, concentrate the cells and add to new medium.

NOTE:

- Cells may decrease in viability as they suspension-adapt. Viability will improve over time.
- After serum-free adaptation, cryopreserve the cells: 10-18 vials (2 x 10^7 cells/mL) per adapted serum level.

VII. Ordering information

Cat. no.	Product	Size
BP12-765Q (US) BEBP02-025Q (EU)	Pro293s™ Chemically Defined Medium-Suspension	1 L
BEBP12-764Q	Pro293a™ Chemically Defined Medium-Adherent	1 L

Product use statement

GMP PRODUCTS ARE INTENDED FOR RESEARCH OR FOR FURTHER MANUFACTURING USE ONLY. This product is not intended for direct therapeutic use in humans.