NeoXP-293

Protein-free Medium

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Chemically Defined Medium for the Growth of 293 cells

Cat. No. CP771

1 X 0.5 L	1 X 1 L	5 X 1 L

(Custom packaging available upon request)

Storage Conditions: 2 to 8°C, in the dark.

Background

NeoXP-293 is a complete, protein-free cell culture medium optimized for growth and recombinant protein or adenovirus production in 293 cells (including 293-F, 293-H) in suspension culture. It is chemically-defined, and contains no components of animal origin. It is supplied as sterile liquid medium in a variety of sizes. It is a ready to use formulation and no additives are required. The medium does not contain antibiotics and these may be added if desired.

Application

NeoXP-293 is a protein-free medium developed for the long-term growth of Human Embryonic Kidney 293 (HEK 293) and related cells. The cells, in a suspension culture, can be subcultured into NeoXP-293 from serum-supplemented media with adaptation.

Quality Control

NeoXP-293 is performance tested in a growth and maintenance assay using 293 cells in a dynamic cell culture system. Additional standard evaluations are pH, sterility, osmolality, and endotoxin.

Physical Conditions

Standard physical conditions for 293 cells grown in NeoXP-293 are 37 ± 0.5°C in a humidified atmosphere of 5% CO_2.

Adaptation of 293 Cells to Protein-Free Culture

Sequential adaptation of 293 cells from serum supplemented to NeoXP-293 may be necessary.

It is critical that cell viability be at least 85% and cells be in the mid-logarithmic phase of growth prior to adaptation. The procedure is as follows:

1. Subculture the cell suspension grown in conventional serum supplemented media into a 50:50 ratio (v/v) of NeoXP-293 and serum supplemented medium at approximately $1x10^5$ cells/mL. Incubate the culture at 37°C in a humidified atmosphere of 5% CO_2. Allow cell density to reach approximately $8x10^5$ cells/mL.
2. Subculture the above into a 75:25 (v/v) mixture of NeoXP-293 and serum supplemented medium. The final cell density should be $4x10^5$cells/mL with at least 85% viability.
3. Continue to subculture the cell suspension in NeoXP-293 at an inoculum of $4x10^5$cells/mL in two more steps of 90:10 and 99:1 (v/v, NeoXP-293: serum supplemented medium). In the final subculturing step the serum concentration will be decreased to 0.1%.
4. Pass the cells in 100% NeoXP-293 every 3-4 days when the cell density reaches 4-8 $x10^5$ cells/mL.
5. After several passages, the cell yield should increase to 1-$3x10^6$ cells/mL after 3-4 days in culture. At this point, the cells are considered to be fully adapted to NeoXP-293.

Cultures may be grown in spinner flasks with impeller speed set at 75-95 rpm or in shake flasks on an orbital shaker platform rotating at 120-135 rpm.

*** Note:** Adaption of cells grown in alternative serum-free or protein-free media other than NeoXP-293 may also be required.

Cryopreservation

1. Harvest cells in the mid logarithmic phase of growth, ≥85% viability.
2. Freeze cells at 1 x 10^7 cells/mL in 40% fresh NeoXP-293, 50% NeoXP-293 conditioned medium (medium in which cells to be frozen have been grown for at least 2 days), 10% DMSO.
3. Use of a controlled rate freezer is recommended to cryopreserve cells in a controlled and reproducible manner. If controlled rate cryopreservation equipment is not available, 293 cells in the described cryogenic storage medium may be preserved using the following protocol:

    a. 1 hour at 4°C
    b. 2 - 4 hours at -20°C
    c. overnight at - 70°C
    d. store in liquid nitrogen

Thawing NeoXP-293 Adapted Cells:

1. Remove vial from Liquid Nitrogen and immediately transfer to 37°C water bath.
2. While holding the tip of the vial, gently agitate the vial, being careful not to allow water to into the vial. Spray thawed vial with 70% ethanol to avoid contamination.
3. When completely thawed, transfer cells to 15mL tube.
4. Slowly add 10mL warm NeoXP-293, mix by inversion, and spin at 1000g for 5min
5. Decant media and resuspend pellet in NeoXP-293.
6. Culture cells in flask on an orbital shaker platform rotating at 120-135 rpm.

Custom Production and Packaging

When you need a unique formulation or special packaging, our Custom Product Services team can modify catalog media formulations and packaging to meet your requirements.

References:

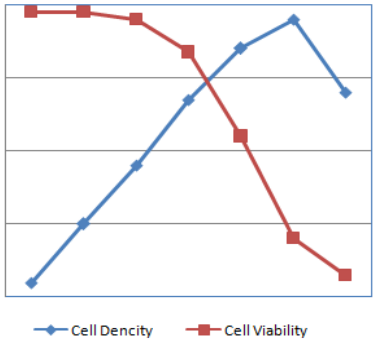
Two Different Serum-free Media and Osmolality Effect Upon Human 293 Cell Growth and Adenovirus Production. Tiago B. Ferreira, Ana L. Ferreira, Manuel J. T. Carrondo and Paula M. AlvesBiotechnology Letters Volume 27, Number 22, 1809-13

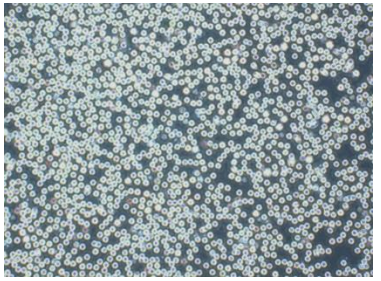
Development and improvement of a serum-free suspension process for the production of recombinant adenoviral vectors using HEK293 cells. Tsao YS, Condon R, Schaefer E, Lio P, Liu Z., Cytotechnology. 2001 Nov;37(3):189-98.

Transient gene expression in mammalian cells grown in serum-free suspension culture. Schlaeger EJ, Christensen K., Cytotechnology. 1999 Jul;30(1-3):71-83.

Adenovirus vector production using low-multiplicity infection of 293 cells., Yamada K, Morishita N, Katsuda T, Kubo S, Gotoh A, Yamaji H., Cytotechnology. 2009 Apr;59(3):153-60. Epub 2009 Jul 8.

CAUTION:
For research use only.
Not intended for human or animal diagnostic or therapeutic uses.





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