HEK|ONE T Chemically Defined Medium for Transient Expression, w/o L-Glutamine, with Growth Hormones

#Cat: NB-58-0040 Size: 1000ml

Product Information

General Information

HEK|ONE T is a chemically defined, animal-component-free medium for transient cells. HEK|ONE T was developed for the cultivation and expression of HEK293 and other mammalian cell lines, with a special focus on transfection applications and virus production. The medium is highly suitable for transient transfection with e.g. polycationic transfection reagents such as polyethylenimine (PEI). HEK|ONE T supports cell growth and production of recombinant proteins and antibodies in suspension culture. It can be used in research and further manufacturing.

Product Specifications

Appearance	Clear yellow orange solution
Formulation	w/o Anti-Clumping Agents
Glucose Concentration	7.0 g/L
Glutamine	No glutamine; supplement with 6 – 8 mM L-glutamine prior to use
Storage and Shelf Life	+2°C to +8°C; protected from light. Please refer to the label for expiry date.
Shipping Conditions	Ambient
Specifications	- Chemically defined - Serum-free - Animal derived component-free - Protein-free

Instructions for Use

Culture Conditions

HEK|ONE T is formulated without L-glutamine. For applications requiring this amino acid, supplement with 6 – 8 mM L-glutamine prior to use. Supplementation of L-glutamine directly to the culture is recommended.

Note: No supplementation with Pluronic® F68 is necessary to maintain cells in suspension.

Cultures should be maintained at +37 °C. For cultivation in an incubator, a 5 % CO2 atmosphere is necessary.

Temperature	+37°C
CO2	5 %
Shaker diameter	5 cm
Shaker speed	125 – 185 rpm

Stepwise adaptation from serum-containing cultures

1. Expand the culture in serum-containing standard medium.
2. Centrifuge a sufficient number of cells for inoculation of suspension culture with 4 – 6 × 10^5 cells/ml at 115 × g for 5 minutes.
3. Resuspend cells in HEK|ONE T (if necessary, include 6 – 8 mM L-glutamine) and 2 % Fetal Bovine Serum (FBS).
4. Passage cells or change medium by centrifugation every two to four days depending on cell density.
5. Reduce serum concentration to 0.5 % after at least three passages.
6. Passage cells or change media by centrifugation every two to four days depending on cell density.
7. Reduce serum concentration to 0 % after two to four passages.
8. Continue cultures until viabilities stabilize at > 90 %.
9. Adapted cells should be inoculated at 2 – 5 × 10^5 cells/ml in HEK|ONE T for optimal performance. Cultures should be diluted every three or four days. Due to aggregation of HEK cells, cultures should be stirred or shaken, using spinner bottles, shaker flasks or similar cultivation systems.

Routine cultivation and cell expansion

1. Pre-equilibrate a sufficient amount of medium in a polycarbonate Erlenmeyer shake flask for 1 hour.
2. Determine viable cell density in the pre-culture.
3. Depending on the inoculation volume, remove the medium from the shake flask to reach the target working volume after inoculation.
4. Seed cells at a target inoculation cell density of 3 × 10^5 cells/ml (operational range 2 – 5 × 10^5 cells/ml).
5. Incubate the culture according to the conditions mentioned in “Culture Conditions”.
6. Routinely passage the culture when viable cell densities between 15 – 40 × 10^5 cells/ml are reached. Typical duration time for the culture is 3 – 4 days.
7. If cell density is too low or cells do not grow in adaption phase, centrifuge the culture and exchange the medium without dilution after 4 days.

Bioreactor cultivation

For best performance the inoculation density in bioreactor should be in the range of 4 – 6 × 10^5 cells/ml in HEK|ONE T. Suggested starting parameters for bioreactor cultivations of HEK cells using HEK|ONE T are pH 7.0 – 7.5, 40 % DO, and a temperature of +37 °C.

Protocol for Transfection

General Guideline

(The setup for transfection can vary depending on the application and cell line. The protocol can be adjusted to optimize the process.)

1. Seed cells one day before transfection to reach 2.5 to 3x10^6 cells/ml on the day of transfection. For best transfection efficiency, cells should have a viability of >95% at the time of transfection.
2. Spin down the cells and resuspend in fresh HEK|ONE T Medium. Conditioned media contains metabolites that can inhibit transfection.
3. For transfection add 2 pg DNA/cell to the culture and gently mix the suspension.
4. Add PEI stock solution in a 1:2 to 1:4 DNA:PEI ratio and mix gently. (Optimal DNA:PEI ratio needs to be evaluated depending on cell line and process. Pre-complexing is not necessary but may lead to higher transfection efficiency.)
5. Incubate the cells for 2-4 h under normal culture conditions.
6. Add 100% fresh HEK|ONE T Medium or 100% HEK|ONE S Medium or 10-50% HEK|ONE Feed and continue cultivation.
7. Measure transfection efficiency after 48 h or continue cultivation until harvest of the product.

Precautions and Disclaimer

This product is for research use and further manufacturing only. Pluronic is a trademark of BASF Corporation.

Help Needed?

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email (info@neo-biotech.com) or phone (+33 9 77 40 09 09).