Neo T-HEK\nChemically Defined Medium for Transient Viral Vector and Recombinant Protein Production, w/o L-Glutamine

#Cat: NB-58-0078 Size: 500ml

General Information

Neo T-HEK is a chemically defined, animal component-free and hydrolysate-free medium for transient transfection of HEK293 cell lines in suspension. It was developed to maximize recombinant protein expression and viral vector replication (e.g., Adeno-associated Virus (AAV) vectors, and Lentivirus (LV) vectors). The medium is particularly suitable for transfection with polymers, such as polyethylenimine (PEI), and transient expression of viral vectors. For viral infections and protein production through stable gene expression, we provide Neo S-HEK (NB-58-0077) in our portfolio.

Product Specifications

Appearance	Clear, light yellow solution
Specifications	- Chemically defined - Serum-free - Animal derived component-free - Hydrolysate-free - Contains no L-Glutamine; supplement with 0.4 g/L 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

Instructions for Use

Important information:

- Neo T-HEK is formulated without L-Glutamine. For applications requiring this amino acid, supplement with 0.4 g/L L-Glutamine prior to use. Supplementation of L-Glutamine directly to the culture is recommended.

Culture Conditions

Temperature	36.5°C
CO_2	7 %
Culture vessel	Shake flask (250 ml)
Shaking rate	155 rpm
Inoculation cell concentration	8×10^5 viable cells/ml

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 – 8 × 10^5 cells/ml at 115 × g for 5 minutes.
3. Resuspend cells in Neo T-HEK (if necessary, include 0.4 g/L 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 8 × 10^5 cells/ml in Neo T-HEK and should be subcultured every three days for optimal performance. 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 2 hour (36.5°C, 7 % CO_2).
2. Inoculate Neo T-HEK with 8 × 10^5 viable cells/ml and subculture every three days for best performance.
3. Incubate the culture according to the conditions mentioned in “Culture Conditions”.
4. Maintain cells in medium at least 3 passages to have full adaptation for optimal performance. Viable cell concentration shall reach at least 30 × 10^5 cells/ml before cell split.
5. 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.

Protocol for Transfection

We offer a Transfection kit for maximal transfection efficiency with PEI prior to viral vector production. The kit includes Neo T-HEK Transfection Enhancer and Neo T-HEK Transfection Booster. Due to their chemically defined and serum-free formulation, both reagents deliver superior transfection efficiency in animal component-free cultivation systems for high titer viral vector production.

Neo T-HEK medium can be used in stock culture, transfection, and production steps.

1. Inoculate the cells with 8 × 10^5 viable cells/ml in a 250 ml shake flask with 50 ml working volume.
2. Two days after inoculation, spin down the cells and resuspend the pellet in pre-warmed fresh Neo T-HEK and dilute the cells to a final density of 30 × 10^5 viable cells/ml. Important note: Conditioned media contain metabolites that can inhibit transfection.
3. Place the cells at 36.5°C with 7 % CO_2 on a shaker
4. Meanwhile, combine DNA at concentration of 1.5 µg/ml and PEI in a vial in a ratio of 1:2 and add immediately 2.25 µl/ml Neo T-HEK Transfection Enhancer. Incubate 8 minutes at room temperature.
5. Add DNA:PEI mixture dropwise to the cells as 10 % of working volume and transfer the flask into the incubator for two hours with shaking.
6. After two hours, add Neo T-HEK Transfection Booster with 62.5 µl/ml of transfection volume and incubate the cells for three days with shaking.
7. Measure transfection efficiency after 48 h or continue cultivation until harvest of the product.

Formulation

This formulation is our proprietary composition and has no counterparts either in its composition, or in its action.

Precautions and Disclaimer

This product is for research use and further manufacturing only.

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).