

**Neo Stable-HEK, chemically defined medium for stable expression,  
w/o L-Glutamine, with Growth Hormones**

#Cat: NB-58-0041      Size: 1000ml

### General Information

Neo Stable-HEK is a complete chemically defined, animal component-free and protein-free medium for stable cell lines. It was developed for high-performance cultivation and expression of HEK293 and other human cell lines. This medium supports cell growth and production of recombinant proteins and antibodies in suspension culture. It is suitable for research and further manufacturing.

### Product Specifications

<b>Appearance</b>	Clear yellow orange solution
<b>Glucose Concentration</b>	7.0 g/L
<b>Glutamine</b>	No glutamine; supplement with 6 – 8 mM L-glutamine prior to use
<b>Storage and Shelf Life</b>	+2°C to +8°C; protected from light. Please refer to the label for expiry date.
<b>Shipping conditions</b>	Ambient
<b>Specifications</b>	<ul style="list-style-type: none"><li>- Chemically defined</li><li>- Serum-free</li><li>- Animal derived component-free</li><li>- Protein-free</li></ul>

### Instructions for Use

#### Culture Conditions

Neo Stable-HEK 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 % CO<sub>2</sub> atmosphere is necessary.

Temperature	37°C
CO <sub>2</sub>	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  $4-6 \times 10^5$  cells/ml at  $115 \times g$  for 5 minutes.
3. Resuspend cells in Neo Stable-HEK (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  $2-5 \times 10^5$  cells/ml in Neo Stable-HEK 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  $3 \times 10^5$  cells/ml (operational range  $2-5 \times 10^5$  cells/ml).
5. Incubate the culture according to the conditions mentioned in "Culture Conditions".
6. Routinely passage the culture when viable cell densities between  $15-40 \times 10^5$  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  $4-6 \times 10^5$  cells/ml in Neo Stable-HEK. Suggested starting parameters for bioreactor cultivations of HEK cells using Neo Stable-HEK are pH 7.0-7.5, 40 % DO, and a temperature of +37 °C.

## 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](mailto:info@neo-biotech.com)) or phone (+33 9 77 40 09 09).