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**Neo Stable-HEK Feed, chemically defined  
feeding supplement, w/o L-Glutamine**  
#Cat: NB-58-0042      Size: 1000ml

### General Information

Neo Stable-HEK Feed is a chemically defined, animal component-free feeding supplement for stable and transient cells. It was developed for the use as feeding solution in combination with Neo Stable-HEK in recombinant protein production as well as Neo Transi-HEX for transient gene expression. The feeding supplement contains highly concentrated nutrients to increase the productivity of HEK293 and other human cells but no lipids, hydrolysates, or growth factors. Neo Stable-HEK Feed supports superior production of recombinant proteins and antibodies in suspension culture by maintaining and extending the production capability of HEK cultures. Consumed substances like vitamins and amino acids are replenished to increase the protein yield by process extension. It is suitable for research and further manufacturing.

### Product Specifications

<b>Appearance</b>	Clear yellow orange solution
<b>Glucose Concentration</b>	40.0 g/L (plus additional carbon source)
<b>Glutamine</b>	No L-glutamine
<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 Feed contains 40 g/L D-glucose plus one additional sugar source and is formulated without L-glutamine. For applications requiring this amino acid, we recommend supplementation of L-glutamine prior to use. L-glutamine can be added during feed preparation or from stock solution directly into the fed-batch cultivation. For higher D-glucose concentrations, D-glucose can be added as well, either during feed preparation or from stock solutions directly into the fed-batch cultivation. 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

### Instructions for fed-batch processes

1. Start the cultivation in batch mode, use one of Neo Stable-HEK media (S or T) as basal medium plus additional L-glutamine as usual.
2. Daily add Neo Stable-HEK Feed including a sufficient amount of D-glucose and L-glutamine or apply additional D-glucose and L-glutamine supplementation to maintain D-glucose levels of 2 – 3 g/L and L-glutamine concentrations of 1 – 3 mM during fed-batch. An exemplary feeding regime for low- and high-consuming cells is shown below. Example of feeding regime in a fed-batch process with low-or high-consuming cells using Neo Stable-HEK basis medium supplemented with 8 mM L-glutamine in 50 ml working volume shaker cultivation:

Time (days)	Low-consuming cells	High-consuming cells
0	0.0 ml	0.0 ml
1	0.0 ml	0.0 ml
2	1.5 ml	1.5 ml
3	2.0 ml	3.0 ml
4	3.0 ml	5.0 ml
5	5.0 ml	5.0 ml
6-end	5.0 ml	6.0 ml

3. Adjust the feeding regime according to the demand of the cell line. Increase feeding with higher growth and cell density or when nutrient limitations occur. Decrease feeding if cells show poor growth, if the pH value is decreasing dramatically, or if the amount of D-glucose is increasing.

### Bioreactor cultivation

For best performance, the inoculation density in bioreactor should be in the range of  $3 - 5 \times 10^5$  cells/mL in Neo Stable-HEK medium. Suggested starting parameters for bioreactor cultivations of HEK cells are pH 7.0 – 7.2, 40 % DO, and a temperature of +37 °C. The cultivation in bioreactor under controlled pH conditions might lead to differences in cellular demands.

Carefully check growth and D-glucose consumption every day. Adjust feeding to higher cell densities by carefully supplementing more Neo Stable-HEK Feed and/or D-glucose and/or L-glutamine in culture in exponential and stationary cultivation phase. Note: Adjustments of cultivation parameters (e.g. pH, pH deadband or temperature) based on your experience and common published values may further improve process performance.

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