Neo S-HEK\nChemically Defined Medium for Stable Virus and Recombinant Protein Production w/o L-Glutamine

#Cat: NB-58-0077 Size: 500ml

General Information

Neo S-HEK is a chemically defined, animal component-free and hydrolysate-free medium for stable HEK293 cell lines in suspension. It was developed for maximal production of viruses (e.g., adenovirus, lentivirus) and recombinant proteins (e.g., antibodies, Fc-fusion proteins). Combined with Neo S-HEK Feed A and Feed B it is suitable for fed-batch processes to reach the full potential of cell growth up to 3×10^7 cells/ml and to increase protein yield.

Neo S-HEK can be used for transfection via electroporation. For transient transfection with polymers (e.g., PEI), we provide Neo T-HEK (NB-58-0078) in our portfolio.

Product Specifications

Appearance	Clear, 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 S-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.
- To obtain your stock culture for example in seed trains and for routinely subculturing the cells it is recommended to supplement the culture volume with 0.25 mg/L insulin (e.g. INS-K). During production phase of recombinant proteins or viruses, no supplementation with insulin is required or recommended.

Culture Conditions

Temperature	36.5°C
CO_2	7 %
Culture vessel	Shake flask (250 ml)
Shaking rate	155 rpm
Inoculation cell concentration	5×10^5 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 – 6 × 10^5 cells/ml at 115 × g for 5 minutes.
3. Resuspend cells in Neo S-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 5 × 105 cells/ml in Neo S-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 hours (36.5°C, 7% CO_2).
2. Inoculate Neo S-HEK (supplemented with insulin, see IMPORTANT INFORMATION above) with 5 × 10^5 viable cells/ml and subculture every three days for best performance.
3. Alternatively, subculture the cells every four days. In this case, inoculate the medium with 3 × 10^5 viable cells/ml. This method gives moderate performance.
4. Incubate the culture according to the conditions mentioned in “Culture Conditions”.
5. Maintain cells in medium at least 3 passages prior to production phase to have full adaptation for optimal performance. Viable cell concentration shall reach at least 25 × 10^5 cells/ml before cell split.
6. If viable cell density (VCD) is too low or cells do not grow in adaption phase, centrifuge the culture and exchange the medium without dilution after 4 days.

Recommended feeding strategy in fed-batch process

Neo S-HEK can be combined with two Feeding supplements Neo S-HEK Feed A and Feed B to produce recombinant proteins or viruses in a fed-batch process. In this process no supplementation of Insulin is required.

1. Growth medium	Neo S-HEK supplemented with 0.25 mg/L insulin
2. Production medium	7 %
3. Feeding Supplement	Neo S-HEK Feed A Neo S-HEK Feed B

Feeding Schedule

Add feeding supplements according to the table below. Feed volume is a percentage [%] of initial culture volume at day 0. For example, 2 % feeding volume for a 50 ml culture corresponds to 1 ml Feeding supplement.

Culture days	0	1	2	3	4	5	6	7	8	9	10	11
Neo S-HEK Feed A to add [% in v/v]			1	2	2	2	2	2	2	2	2	2
Neo S-HEK Feed B to add [% in v/v]			0.2	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4

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).