

## **Product Information**

**HEKventure S** 

Chemically Defined Medium for Stable Virus and Recombinant Protein Production w/o L-Glutamine, sterile-filtered

Cat. No. HEKVS-500ML (500 ml)

#### **General Information**

HEKventure S 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 HEKventure Feed A and Feed B it is suitable for fed-batch processes to reach the full potential of cell growth up to 3 × 10<sup>7</sup> cells/ml and to increase protein yield.

HEKventure S can be used for transfection via electroporation. For transient transfection with polymers (e.g., PEI), we provide HEKventure T (HEKVT-500ML) in our portfolio.

### **Product Specifications**

Appearance	Clear, yellow solution
Specifications	<ul> <li>Chemically defined</li> <li>Serum-free</li> <li>Animal derived component-free</li> <li>Hydrolysate-free</li> <li>Contains no L-Glutamine; supplement with 0.4 g/L L-Glutamine prior to use</li> </ul>
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:

- HEKventure S 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 <sub>2</sub>	7 %
Culture vessel	Shake flask (250 ml)
Shaking rate	155 rpm
Inoculation cell concentration	5 × 10 <sup>5</sup> 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  $4 6 \times 10^5$  cells/ml at 115 × g for 5 minutes.



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- 3. Resuspend cells in HEKventure S (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 %.
- Adapted cells should be inoculated at 5 × 10<sup>5</sup> cells/ml in HEKventure S 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<sub>2</sub>).
- 2. Inoculate HEKventure S (supplemented with insulin, see IMPORTANT INFORMATION above) with  $5 \times 10^5$  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<sup>5</sup> 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<sup>5</sup> 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

HEKventure S can be combined with two Feeding supplements HEKventure 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	HEKventure S supplemented with 0.25 mg/L insulin					
2. Production medium	HEKventure S without insulin					
3. Feeding Supplement	HEKventure Feed A HEKventure 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
HEKventure Feed A to add [% in v/v]			1	2	2	2	2	2	2	2	2	2
HEKventure 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



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### **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 (techservice@capricorn-scientific.com) or phone (+49 6424 944640).