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## 1. Product description

### Components and specifications

HEK FS  
(for preparation of liquid media from powder  
see Solubilization Protocol)

with 40 g/L D-glucose  
without L-glutamine

Chemically defined  
Free of animal-derived components  
Free of proteins  
Free of growth factors

### Storage

Store protected from light at 2–8 °C. Do not freeze.

### Intended use

Intended for *in vitro* research and manufacturing processes **only**. Do not use for injection or infusion!

## 2. Background information and applications

HEK FS is a chemically defined, animal component-free medium supplement. It is developed for the use as feeding solution in combination with HEK GM in recombinant protein production as well as HEK TF for transient gene expression. The feed supplement contains highly concentrated nutrients to increase the productivity of HEK and other human cells but no lipids, hydrolysates, or growth factors. The 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.

## 3. Protocol

### 3.1 Preparations

All procedures should be carried out using sterile techniques in a biosafety cabinet.

The HEK FS 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.

### 3.2 Culture conditions

Cultures should be maintained at 37 °C. For cultivation in an incubator, a 5% CO<sub>2</sub> atmosphere is necessary.

Parameter	Value[-]
Shaker diameter	5 cm
Shaker speed	125-185 rpm
Temperature	37°C
CO <sub>2</sub>	5%

**Table 1:** Recommended culture conditions for use of Xell media and feed products.

Using the set-up listed in table 1, the working volume of different shake flask sizes was determined (table 2). For cell lines with a strong aggregation, baffled shakers may be used. For this setup, a reduction of the shaking speed might be necessary.

Size of shaker [mL]	Shape [-]	Working volume [mL]
125	plain, vent cap	20 - 50
250	plain, vent cap	80 - 150
500	plain, vent cap	200 - 300
1000	plain, vent cap	400 - 600

**Table 2:** Recommended culture working volumes for use of Xell media and feed products in various shake flask sizes.

### 3.3 Instructions for use in fed-batch

- 1) Start the cultivation in batch mode, use one of Xell's media products and L-glutamine as usual.
- 2) Daily add HEK FS 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 in table 3.

Process time [days]	HEK FS per 50 mL medium	
	Low-consuming cells	High-consuming cells
0	0 mL	0 mL
1	0 mL	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

**Table 3:** Example of feeding regime in a fed-batch process with low- or high-consuming cells using Xell's basis medium supplemented with 8 mM L-glutamine in 50 mL working volume shaker cultivation.

- 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.

### 3.4 Bioreactor cultivation

For best performance, the inoculation density in bioreactor should be in the range of  $3\text{-}5 \cdot 10^5$  cells/mL in Xell medium. Suggested starting parameters for bioreactor cultivations of HEK cells using Xell medium 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 HEK FS 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.*

## 4. Ordering information

Product	Application	Order No.
HEK GM	base medium and protein production for various human cell lines, e.g. HEK cells	851
HEK TF	transfection medium, suitable for growth, transfection/infection and production for various HEK cells and other human cell lines; esp. for transfection	861
HEK ViP NB	Transfection and production medium for HEK cells with a <b>basic nutrient</b> level	891
HEK ViP NX	Transfection and production medium for HEK cells with a <b>high nutrient</b> level	892
Basic Feed	feed supplement for CHO, HEK, hybridoma, et al.	1092
HEK FS	Feed supplement for various human cell lines, e.g. HEK	871

**Table 4:** HEK products by Xell

Place orders: [order@xell.de](mailto:order@xell.de)

For further information or assistance contact us.

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