Customized solutions for cell cultures.

Protocol for use
HEK FS_2
Order No. 880

Contents

1. Product description

Components and specifications

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_2 is a chemically defined, animal component-free medium supplement. It is developed for the use as feeding solution e.g. in virus/viral vector production, recombinant protein production as well as 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 e.g. 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.

Note: HEK FS_2 does not support transfection; in transient transfection applications, add HEK FS_2 only after transfection (from 4 h post transfection).

3. Protocol

3.1 Preparations

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

The HEK FS_2 contains 40 g/L D-glucose plus one additional sugar source and is formulated without L-glutamine. A supplementation of L-glutamine prior to use or separate feeding with glutamine may be required. 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_2 atmosphere is necessary.

Parameter	Value[-]
Shaker throw	5 cm
Shaker speed	125-185 rpm
Temperature	37°C
CO_2	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

Start the cultivation in batch mode, use one of Xell's media products and L-glutamine as usual. Depending on your process and cell line you might want to choose between the two exemplary feeding options. Further feeding adjustments might benefit your process.

Option 1: short-term/transient production of e.g. viral vectors:

Add HEK FS_2 once during production phase, e.g. 4-6 h or 24 h post transfection. Use at 10 % of final culture volume (recommended range: 5-15 %).

Option 2: stable/longer production process (e.g. for mAb)

Daily, add HEK FS_2 including a sufficient amount of D-glucose and L-glutamine and/or apply additional D-glucose and L-glutamine supplementation to maintain proper D-glucose levels and L-glutamine concentrations during fed-batch. An exemplary feeding regime for low- and high-consuming cells is shown in table 3.

Process time [days]	HEK FS_2 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.

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 $3–5 \cdot 10^5$ 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_2 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, temperature or stirring setup) based on your experience and common published values may further improve process performance.

For further information or assistance contact us.

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