Customized solutions for cell cultures.

Protocol for use
BHK FS
Order No. 915

Contents

1. Product description

Components and specifications

BHK FS
(for preparation of liquid feed from powder see Solubilization Protocol)

with 20 g/L D-glucose
without L-glutamine
without hypoxanthine/thymidine

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

The BHK FS is a chemically defined, animal component-free medium supplement. It is developed for the use as feeding solution in large scale virus and recombinant protein production. The feed supplement contains highly concentrated nutrients to increase the productivity of cultured cells but no lipids, hydrolysates, or growth factors. BHK FS supports superior production of viruses and recombinant proteins in suspension culture by increasing the final cell density and extending the production capability of the cultures compared to batch process. Consumed substances like vitamins and amino acids are replenished to extend the process and thereby increase product yield.

3. Protocol

3.1 Preparations

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

The BHK FS contains 20 g/L D-glucose 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_2 atmosphere is necessary.

Parameter	Value[-]
Shaker diameter	5 cm
Shaker speed	110-185 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

1) Start the cultivation in batch mode, use one of Xell's media products and L-glutamine as usual.

2) Daily add BHK 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]	BHK FS per 50 mL medium	
	Low-consuming cells	High-consuming cells
0	0 mL	0 mL
1	0 mL	0 mL
2	1 mL	1.5 ml
3	1 mL	1.5 ml
4	1.5 mL	2.5 mL
5	1.5 mL	2.5 mL
6 - end	2 mL	3.5 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 4–6×10^5 cells/mL in Xell medium. Suggested starting parameters for bioreactor cultivations of e.g. BHK cells using Xell medium are pH 7.0–7.2, 30-40% 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 BHK FS and/or D-glucose and/or L-glutamine in culture in exponential and stationary cultivation phase.

4. Ordering information

Product	Application	Order No.
BHK Medium	base medium and protein production for BHK cells	910
BHK FS	feed supplement for BHK cells	915

Table 4: BHK products by Xell

Place orders: order@xell.de

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