

Contents

1. Product description
2. Background information and applications
3. Protocols
 - 3.1 Preparations
 - 3.2 Culture conditions
 - 3.3 Instructions for use in fed-batch
 - 3.4 Bioreactor cultivation

1. Product description

Components and specifications

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

with 20 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

HYB FS is a chemically-defined and animal component-free medium supplement. It is developed for the use as a feeding solution for antibody production. The feed supplement contains highly concentrated nutrients, but no lipids, hydrolysates, or growth factors. The feed supports superior production of antibodies in suspension culture by maintaining and extending the production capability of hybridoma cultures. Consumed substances like vitamins and amino acids are replenished to increase the protein yield.

3. Protocol

3.1 Preparations

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

HYB FS contains 20 g/L D-glucose and is formulated without L-glutamine. A supplementation of L-glutamine prior to use is 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% CO₂ atmosphere is necessary.

Parameter	Value[-]
Shaker diameter	5 cm
Shaker speed	125-185 rpm
Temperature	37°C (36.5°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 sensitive cell lines it is recommended to use a working volume near to the indicated maximum filling and to keep the shaker speed at 125 - 150 rpm.

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 HYB FS 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 of during fed-batch. An exemplary feeding regime for low- and high-consuming cells is shown in table 3.

* L-glutamine should be added directly to the process before application of HYB FS, since L-glutamine is unstable by nature even at recommended storage conditions of HYB FS.

Process time [days]	HYB FS per 50 mL medium	
	Low-consuming cells	High-consuming cells
0	0 mL	0 mL
1	2.0 mL	1.5 mL
2	4.0 mL	1.5 ml
3	6.0 mL	3.0 ml
4	8.0 mL	6.0 mL
5	8.0 mL	6.0 mL
6 - end	8.0 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 6-8 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.

Note: For some hybridoma cell lines simple feeding strategies like e.g. daily addition of 50 mL HYB FS per L of culture from 24 h on or one-time bolus feeding of about 15 - 30% (v/v) after 48 h of cultivation was found to be beneficial.

3.4 Bioreactor cultivation

For best performance the inoculation density in bioreactor should be in the range of $2-4 \times 10^5$ cells/mL in Xell medium. Suggested starting parameters for bioreactor cultivations of e.g. hybridoma cell lines using Xell medium are pH 7.0-7.2, 30% DO, and a temperature of 37 °C (36.5 °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 HYB 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.

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