

Contents

1. Product description
2. Background information and applications
3. Protocols
 - 3.1 Preparations
 - 3.2 Culture conditions
 - 3.3 Instructions for use
 - 3.3.1 Thawing of cells
 - 3.3.2 Routine cultivation and cell expansion
 - 3.3.3 Stepwise adaption from serum-containing cultures
 - 3.3.4 Bioreactor cultivation
 - 3.3.5 Freezing of cells

1. Product description

Components and specifications

HYB GM medium
(for preparation of liquid media from powder see Solubilization Protocol)

without L-glutamine
without growth hormone

Chemically defined
Free of animal-derived components
Free of proteins

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 GM is a completely chemically-defined, animal component-free and protein-free medium. HYB GM was developed by Xell for high-performance cultivation of hybridoma cell lines. HYB GM supports cell growth and production of antibodies in suspension culture. It can be used in research or in manufacturing applications.

3. Protocols

3.1 Preparations

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

HYB GM is formulated without L-glutamine. Supplementation with 6-8 mM L-glutamine prior to use is recommended.

Note: No supplementation with a protectant e.g. Pluronic® F68 is necessary to maintain cells in suspension.

3.2 Culture conditions

Cultures should be maintained at 36.5°C to 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
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 polycarbonate Erlenmeyer shake flask sizes was determined (table 2). For cell lines with strong aggregation, baffled shake flasks 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

3.3.1 Thawing of cells

- 1) Quickly thaw a vial of frozen cells in a 37 °C water bath.
- 2) Transfer the cells aseptically to a centrifugation tube containing 10 mL of HYB GM medium.
- 3) Centrifuge cell suspension at 115×g for 5 minutes.
- 4) Aspirate supernatant completely and discard.
- 5) Resuspend the cells in 10 mL HYB GM medium per vial.

- 6) Adjust viable cell density to $4\text{-}8 \times 10^5$ cells/mL by medium addition and transfer cell suspension into an agitated or stationary cultivation system (e.g. T-75 tissue culture flask, 125 mL polycarbonate Erlenmeyer flask, or 50 mL filter tube).
- 7) Count the cells after 24-48 hours for assessment of cell density and viability.
- 8) Adjust cell density to $3\text{-}5 \times 10^5$ cells/mL. *
- 9) Proceed with routine cultivation.

* Depending on the cell line, the target inoculation cell density can be lower.

3.3.2 Routine cultivation and cell expansion

- 1) Pre-equilibrate a sufficient amount of medium in a polycarbonate Erlenmeyer shake flask (Parameters listed in tables 1 and 2) for 1 hour. **
- 2) Determine viable cell density in the pre-culture.
- 3) Depending on the inoculation volume, remove medium from the shake flask to reach the target working volume after inoculation. Final working volume of given shaker size is listed in table 2.
- 4) Seed cells at a target inoculation cell density of 2×10^5 cells/mL (operational range 1.5- 3×10^5 cells/mL).
- 5) Incubate the culture according to the conditions listed in table 1.
- 6) Routinely passage the culture when viable cell densities between $10\text{-}20 \times 10^5$ cells/mL are reached. Typical duration time for the culture is 2-3 days. It is recommended to maintain cells in 2-2-3 day subcultivation procedure, while cells are seeded at the lower end of the cell density range before 3 days of incubation.
- 7) If cell density is too low or cells do not grow in adaption phase, centrifuge the culture and exchange the medium without dilution after 3 days.

** Depending on cell line, the pre-equilibration of medium might be not necessary. For some cell lines the use of 2-8°C cold culture medium directly from refrigerator was found to be beneficial. This procedure eliminates handling variations of the medium in the pre-equilibration phase of the medium.

3.3.3 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 $2\text{-}4 \times 10^5$ cells/mL at $115 \times g$ for 7 minutes.
- 3) Resuspend cells in Xell medium (if necessary, include 6-8 mM 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 %.

- 9) Adapted cells should be maintained according to instructions for routine cultivation to reach optimal performance. Due to high oxygen demand of hybridoma cell lines, cultures should be stirred or shaken, using spinner bottles, shaker flasks or similar cultivation systems.

Depending on the cell line direct adaption from serum containing culture to HYB GM medium might be possible as well.

3.3.4 Bioreactor cultivation

For best performance the inoculation density in bioreactor should be in the range of $2\text{-}4 \times 10^5$ cells/mL in Xell medium. Suggested starting parameters for bioreactor cultivations of hybridoma cell lines using Xell medium are pH 7.2-7.7, 40% DO, and a temperature of 37 °C (36.5°C). Please reduce bubbling by submersive aeration and resulting foam formation to a necessary minimum during initial phase of the process.

Note: No supplementation with e.g. Pluronic® F68 is necessary to maintain cells in suspension.

3.3.5 Freezing of cells

Cells can be frozen in HYB GM medium without the use of serum.

- 1) Choose a well-growing culture with viabilities above 90 %.
- 2) Prepare a freezing medium consisting of 90 % HYB GM and 10 % dimethyl sulfoxide (DMSO; cell culture grade).
- 3) Cool down the freezing medium to 2-8 °C.
- 4) Centrifuge the cells at $115 \times g$ for 7 minutes.
- 5) Aspirate supernatant completely.
- 6) Resuspend the cells in freezing medium at 1×10^7 cells/mL.
- 7) Rapidly transfer 1.5 mL of this suspension to sterile cryovials.
- 8) Place the vials in a pre-cooled (2-8 °C) freezing module and store the modules including the vials for 24 hours at -80 °C.
- 9) Transfer the cryovials to a -140 °C to -196 °C system for long time storage.

For further information or assistance contact us.

www.xell.de
info@xell.de

Xell AG
 Alte Verler Str. 1
 33689 Bielefeld
 Germany

Fon: +49 (0)521 96989-200
 Fax: +49 (0)521 96989-201

