Customized solutions for cell cultures.

Protocol for use
HEK GM
Order No. 851

Contents

1. Product description

Components and specifications

HEK GM medium

without L-glutamine
with 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

HEK GM is intended for research or further manufacturing purposes only. It is not intended for human or animal diagnostic or therapeutic use.

2. Background information and applications

HEK GM is a complete chemically-defined, animal-component-free and protein-free medium. HEK GM was developed by Xell for high-performance cultivation of HEK and other human cell lines. HEK GM supports cell growth and production of recombinant proteins and 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.

HEK GM medium is formulated without L-glutamine. For applications requiring this amino acid, supplement with 6-8 mM L-glutamine prior to use. Supplementation of L-glutamine directly to the culture is recommended.

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

3.2 Culture conditions

Cultures should be maintained at 37 °C. For cultivation in an incubator, a 5% CO_2 atmosphere is necessary.

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

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

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.

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

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

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 HEK 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 HEK GM medium per vial.
6) Adjust viable cell density to 5-10·10^5 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 3–6·10^5 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 3·10^5 cells/mL (operational range 2–5·10^5 cells/mL).
5) Incubate the culture according to the conditions listed in table 1.
6) Routinely passage the culture when viable cell densities between 15–40·10^5 cells/mL are reached. Typical duration time for the culture is 3-4 days.
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 4 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 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 4–6·10^5 cells/mL at 115×g for 5 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 inoculated at 2-5·10^5 cells/mL in Xell medium for optimal performance. Cultures should be diluted every three or four days. Due to aggregation of HEK cells, cultures should be stirred or shaken, using spinner bottles, shaker flasks or similar cultivation systems.

3.3.4 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 HEK cells using Xell medium are pH 7.0–7.2, 40% DO, and a temperature of 37 °C.

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 the HEK 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% HEK GM medium and 10 % dimethyl sulfoxide (DMSO; cell culture grade).
3) Cool down the freezing medium to 2–8 °C.
4) Centrifuge the cells at 115×g for 5 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.

4. Ordering information

Table 3: HEK Products by Xell.

Product	Application	Order No.
HEK GM	Growth and production	851-0001 (1000 mL)
HEK TF	Growth and transient gene expression	861-0001 (1000 mL)
HEK FS	Nutrient supplement for HEK cultivation	871-0001 (1000 mL)

Place orders: order@xell.ag

5. References

Beckmann *et al*. BMC Proceedings 2015, 9(Suppl 9):P27

Püngel *et al*. BMC Proceedings 2015, 9(Suppl 9):P18

Püngel *et al*. BMC Proceedings 2013, 7(Suppl 6):P27

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