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## 1. Product description

### Components and specifications

HEK TF medium  
(for preparation of liquid media from powder  
see Solubilization Protocol)

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

Intended for *in vitro* research and manufacturing processes **only**. Do not use for injection or infusion!

## 2. Background information and applications

HEK TF is a complete chemically-defined, animal-component-free medium. HEK TF was developed by Xell for cultivation of HEK and other mammalian cell lines, with a special focus on transfection applications and virus production. The medium is especially suited for transient transfection with e.g. polycationic transfection reagents such as polyethylenimine (PEI). HEK TF supports cell growth and production of e.g. 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 TF is formulated without L-glutamine. Supplementation with 6-8 mM L-glutamine prior to use 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<sub>2</sub> atmosphere is necessary.

Parameter	Value[-]
Shaker diameter	5 cm
Shaker speed	125-185 rpm
Temperature	37°C
CO <sub>2</sub>	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 HEK TF.
- 3) Centrifuge cell suspension at 115×g for 5 minutes.
- 4) Aspirate supernatant completely and discard.
- 5) Resuspend the cells in 10 mL HEK TF per vial.
- 6) Adjust viable cell density to 5-10×10<sup>5</sup> 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  $3-6 \times 10^5$  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  $3 \times 10^5$  cells/mL (operational range  $2-5 \times 10^5$  cells/mL).
- 5) Incubate the culture according to the conditions listed in table 1.
- 6) Routinely passage the culture when viable cell densities between  $15-40 \times 10^5$  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 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  $4-6 \times 10^5$  cells/mL at  $115 \times g$  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  $2-5 \times 10^5$  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  $4-6 \times 10^5$  cells/mL in Xell medium. Suggested starting parameters for bioreactor cultivations of HEK cells using Xell medium are pH 7.0-7.5, 40% DO, and a temperature of 37 °C. The medium already contains Pluronic® F68, further supplementation is not necessary.

*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 HEK TF 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 TF 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 5 minutes.
- 5) Aspirate supernatant completely.
- 6) Resuspend the cells in freezing medium at  $1 \times 10^7$  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.

### 3.3.6 Transfection of cells

HEK TF has been developed to especially support transfection applications and allows high transient gene expression.

The setup for transfection can vary depending on the application and cell line used. It is advisable to use established protocols or test different protocols to reach optimum performance.

As a basic guideline, the following protocol can be applied for small scale transfections:

- 1) One day before transfection, seed cells with an appropriate inoculum to reach  $3 \cdot 10^6$  cells/mL on the day of transfection.
- 2) Before transfection, spin down cells and resuspend in fresh HEK TF. The cell culture should have high viability.

Alternatively to 1) and 2), transfect cells at  $3 \cdot 10^6$  cells/mL without prior resuspension in fresh medium or dilute culture to the required cell density on the day of transfection.

Note: Depending on cells and protocol, the presence of spent medium can lower transfection efficiency.

- 3) For transfection, add 2 µg DNA/cell to the culture and gently mix the suspension.
- 4) Add e.g. PEI or PEI-MAX (Polysciences) stock solution in a 1:2 to 1:4 (w/w) DNA:PEI ratio and gently mix. Optimal DNA:PEI ratio may vary depending on cell line and transfection reagent and needs to be evaluated.

Note: No pre-incubation of medium and PEI or medium and DNA is necessary for complex formation. Nevertheless, pre-complexing may lead to higher transfection efficiency.

- 5) Incubate cells under normal cultivation conditions for 2-4 h.
- 6) Add 100% fresh HEK TF and continue cultivation.  
or: Add 100% HEK GM and continue cultivation.  
or: Add 10-50% HEK FS and continue cultivation.

Note: Fresh medium or feed can be added several times during subsequent cultivation. Add as needed.

- 7) Perform analysis after 48 h to measure transfection efficiency or continue cultivation until harvest of product.

Transfection efficiencies with optimal protocol should be higher than 80% for various cell lines. Otherwise, adjust protocol to improve process performance.

## 4. 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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