Customized solutions for cell cultures.

Protocol for use
HEK ViP NB
Order No. 891

Contents

1. Product description

Components and specifications

without L-glutamine
without growth hormone
with HT (hypoxanthine/thymidine)

Chemically defined
Free of animal-derived components
Free of proteins and hydrolysates

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 ViP NB is a complete chemically-defined, animal-component-free medium. HEK ViP NB was developed by Xell for cultivation of HEK and other mammalian cell lines, with a special focus on transfection applications and optimized for virus/viral vector production. The medium is especially suited for transient transfection with e.g. polycationic transfection reagents such as polyethylenimine (PEI) and other commercial reagents. HEK ViP NB supports cell growth and production of e.g. proteins or viral vectors in suspension culture in various scales. 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 ViP NB is formulated without L-glutamine. Supplementation with 6-8 mM L-glutamine prior to use is recommended. For cell lines requiring the addition of growth factors, supplement accordingly (e.g. 5-10 mg/L rInsulin).

Note: No supplementation with e.g. Poloxamer 188 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.

Parameter	Value[-]
Shaker throw	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 setup 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 or in hand.
2) Transfer the cells aseptically to a centrifugation tube containing 20 mL of HEK ViP NB.
3) Centrifuge cell suspension at 200×g for 5 minutes.
4) Aspirate supernatant completely and discard.

5) Resuspend the cells in appropriate volume of HEK ViP NB to reach the desired cell density (e.g 3-6×10^5 cells/mL).
6) Transfer cell suspension into an agitated cultivation system (e.g. 125 mL polycarbonate Erlenmeyer flask, or 50 mL filter tube) and place into incubator.
7) Count the cells after 3-4 days for assessment of cell density and viability.
8) Adjust cell density to 3–6×10^5 cells/mL. *
9) Proceed with routine cultivation and passaging.

* 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 3–5×10^5 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 adaptation phase, centrifuge the culture and exchange the medium without dilution after 3-4 days.

** Depending on cell line, the pre-equilibration of medium might not be 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 adherent 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 200×g for 5 minutes. Discard supernatant.
3) Resuspend cells in Xell medium (if necessary, include 6-8 mM L-glutamine and/or growth factor) 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 viability stabilizes at > 90% and growth rates remain constant over 3-5 passages.
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 Direct adaptation from serum-containing adherent 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 200×g for 5 minutes. Discard supernatant.
3) Resuspend cells in Xell medium (if necessary, include 6-8 mM L-glutamine and/or growth factor) without fetal bovine serum (FBS).
4) Passage cells or change medium by centrifugation every two to four days depending on cell density.
5) Continue cultures until viability stabilizes at > 90% and growth rates remain constant over 3-5 passages.
6) 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.

Further tips on adaptation can be found here: Adaptation of mammalian cell lines to serum-free culture media | Xell AG

3.3.5 Direct adaptation from suspension cultures in chemically defined media

1) Expand the culture in chemically defined standard medium.
2) Centrifuge a sufficient number of cells for inoculation of suspension culture with 4–6×10^5 cells/mL at 200×g for 5 minutes. Discard supernatant.
3) Resuspend cells in Xell medium (if necessary, include 6-8 mM L-glutamine and/or growth factor).
4) Passage cells or change medium by centrifugation every two to four days depending on cell density.
5) Continue cultures until viability stabilizes at > 90% and growth rates remain constant over 3-5 passages.
6) 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.

3.3.6 Bioreactor cultivation

For best performance, the inoculation density in bioreactor should be in the range of 3–6×10^5 cells/mL in Xell medium. Suggested starting parameters for bioreactor cultivations of HEK cells using Xell medium are pH 7.0–7.2, 30-40% DO, and a temperature of 37 °C. The medium already contains a poloxamer as surfactant, further supplementation is not necessary.

Note: No supplementation with Poloxamer 188 is necessary to maintain cells in suspension.

3.3.7 Freezing of cells

Cells can be frozen in HEK ViP NB 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 ViP NB and 10 % dimethyl sulfoxide (DMSO; cell culture grade).
3) Cool down the freezing medium to 2–8 °C.
4) Centrifuge the cells at 200×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 term storage.

3.3.8 Exemplary transfection protocol

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

The setup for transfection can vary depending on the application, transfection reagent and cell line used. It is advisable to use established protocols or test different protocols to reach optimum performance. Transfection efficiencies with optimal protocol should be higher than 80% 72 h after transfection for most cell lines. Otherwise, adjust protocol to improve process performance.

As a basic guideline, the following exemplary protocol can be applied for transfection with PEI-MAX (Polysciences). Other commonly used transfection reagents work well in combination with Xell's media using a similar setup and pre-complexation in medium. Cultures can be supplemented with growth medium or feed about 2-4 h post-transfection or during the production phase (e.g. 24-48 h post-transfection), if necessary.

1) One day before transfection, seed cells with an appropriate inoculum to reach e.g. 3×10^6cells/mL on the day of transfection.
2) Optionally: on day of transfection, add 20-50 % fresh medium to the culture.
3) Pre-complexing:
   a. Prepare 1 tube with 5-10 % of total media volume.
   b. Add DNA and PEI to the respective tube (e.g. PEI:DNA ratio 4:1, 1 µg DNA per $1x10^6$ cells/mL) and invert tube for 20 s with 15 rpm on an overhead rotator.
   c. Incubate for 20-30 min at room temperature.
   d. Add transfection mix to the freshly diluted culture (see 2).

Note: After successful testing, PEI and DNA can be added to freshly diluted culture directly.

4) Place cultures in the incubator to start the production phase.
5) Determine transfection efficiency (e.g. via GFP), virus titer, etc. at appropriate time points post-transfection (e.g. 24-72 h post-transfection for transfection).

For further tips and troubleshooting, please contact us directly.

For further information or assistance contact us.

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