Customized solutions for cell cultures.

Protocol for use
HEK ViP NX
Order No. 892

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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 NX is a complete chemically-defined, animal-component-free medium. HEK ViP NX 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). HEK ViP NX supports cell growth and production phase 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 NX is formulated without L-glutamine. Supplementation with 6-8 mM L-glutamine prior to use is recommended.

Note: No supplementation with e.g. poloxamers such as 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.

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 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.
2) Transfer the cells aseptically to a centrifugation tube containing 10 mL of HEK ViP NX.
3) Centrifuge cell suspension at 115×g for 5 minutes.
4) Aspirate supernatant completely and discard.
5) Resuspend the cells in 10 mL HEK ViP NX per vial.
6) Adjust viable cell density to 5-10×10^5 cells/mL by medium addition and transfer cell suspension into an

agitated cultivation system (e.g. 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 3–6×10⁵ 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 $3–5\times10^5$ cells/mL).
5) Incubate the culture according to the conditions listed in table 1.
6) Routinely passage the culture when viable cell densities between $15–40\times10^5$ 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 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 adherent cultures

1) Expand the culture in serum-containing standard medium.
2) Centrifuge a sufficient number of cells for inoculation of suspension culture with 4–6×10⁵ cells/mL at 115×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 2-5×10⁵ 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 4–6×10⁵ cells/mL at 115×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 2-5×10⁵ 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.

3.3.5 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 4–6×10⁵ cells/mL at 115×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 2-5×10⁵ 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 3–6×10⁵ 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 poloxamers such as Pluronic® F68 is necessary to maintain cells in suspension.

3.3.7 Freezing of cells

Cells can be frozen in HEK ViP NX 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 NX 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.

3.3.8 Exemplary transfection protocols

HEK ViP NX has been developed to especially support transfection applications and allow 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. Transfection efficiencies with optimal protocol should be higher than 80% for most cell lines. Otherwise, adjust protocol to improve process performance.

As a basic guideline, the following exemplary protocols can be applied for small scale (fresh medium) or scale-up (spent medium) transfections. Protocols can be applied either with or without pre-complexing of DNA with transfection reagent (e.g. PEI). Pre-complexing can be done directly in HEK ViP NX medium.
Cultures can be supplemented with growth medium or feed about 2-4 h post-transfection, if necessary.

3.3.8.1 Transfection in small scale (fresh medium)

1) One day before transfection, seed cells with an appropriate inoculum to reach e.g. $3\cdot10^6$ cells/mL on the day of transfection.
2) Before transfection, spin down cells and resuspend in fresh HEK VIP NX. The cell culture should have a high viability (> 90%).
3) For transfection, add 2 pg 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 ViP NX 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.

3.3.8.2 Transfection in spent medium (scale-up)

1) One day before transfection, dilute cells 1:2 (desired viable cell density between 1-1.5 $\cdot10^6$ cells/mL)
2) On day of transfection, dilute cells again 1:2 directly before transfection (desired viable cell density between 1-1.5 $\cdot10^6$ cells/mL)
3) Pre-complexing:
   a. Prepare 2 tubes with each 4 % of total media volume (e.g. 32 µL for 8 mL total culture volume per transfection reaction).
   b. Add PEI and DNA to the respective tubes (PEI:DNA ratio 4:1, e.g. 32 µg PEI and 8 µg DNA for 8 mL total culture volume per transfection reaction) and vortex for 60 sec.
   c. Add PEI-medium mix to DNA-medium mix and vortex for additional 60 sec.
   d. Incubate for 15 min at room temperature.
   e. 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 for starting the production phase.
5) Determine transfection efficiency (e.g. via GFP), virus titer, etc. at appropriate time points post-transfection (e.g. 48 h post-transfection for transfection efficiency determination of GFP).

Note: Xell routinely performs pre-complexing for spent media protocol.

Note: Spent media protocol is easily scalable and therefore allows for large-scale transfection, e.g. in bioreactors.

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