Customized solutions for cell cultures.

Protocol for use
adHEK
Order No. 867

Contents

1. Product description

Components and specifications

without L-glutamine
without phenol red
with HT (hypoxanthine/thymidine)

Chemically defined
Free of animal-derived components
Free of hydrolysate

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

adHEK is a complete chemically defined, animal-component-free and hydrolysate-free medium. It was developed by Xell for high-performance cultivation of adherent HEK and other human cell lines. adHEK supports cell growth and production of e.g. recombinant proteins and antibodies in adherent culture. It can be used in research or in manufacturing applications.

- Little or no adaptation required from other serum-free media
- Easy adaptation from serum-containing base media
- Supports stable growth of adherent cells at high viability
- Approved by ISO 9001 QMS (Quality Statement)

3. Protocols

3.1 Preparations

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

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

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[-]
Temperature	37°C
CO_2	5%

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

Size of culture flask [cm2]	Working volume [mL]
25	5-10
75	25
150	30

Table 2: Recommended culture working volumes for use of Xell media in various culture 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 adHEK.
3) Centrifuge cell suspension at 200×g for 5 minutes.
4) Aspirate supernatant completely and discard.
5) Resuspend the cells in 10 mL adHEK per vial.
6) Adjust the viable cell density to the required cell density (e.g. 1×10^5 cells/mL) by medium addition and transfer cell culture into the cultivation system.
7) Optional: Add 1-2% FCS, e.g. if cells are subjected to step-wise serum withdrawal.
8) View the culture under the microscope after 48 hours for assessment of viability and confluence. Optional: Count the cells after 24 hours for assessment of cell density and viability.
9) Proceed with routine cultivation.

3.3.2 Routine cultivation and cell expansion

1) Pre-equilibrate a sufficient amount of medium in a 25 cm^2 (adjust volumes for larger culture vessels) tissue culture – treated surface culture flask or coated culture flask e.g. with poly-l-lysine or fibronectin (Parameters listed in tables 1 and 2) for 1 hour. *
2) Thaw Accutase®** at room temperature with regular gentle swirling to ensure thermal homogeneity (do not thaw at 37°C).
3) Aspirate the medium and wash with 3 mL of DPBS without calcium and magnesium.
4) Add Accutase® to culture flask using aseptic procedures at 3 mL per 25 cm^2 surface area.
5) Return culture to 37°C incubator and allow cells to detach 5–10 minutes.
6) Determine viable cell density and passage at the target inoculation cell density. No additional washes or enzyme inhibitors are required.
7) Incubate the culture according to the conditions listed in table 1.
8) Routinely passage the culture if the confluence of the culture is < 80% (typical duration time for the culture is 3-4 days).
9) If cell density is too low or cells do not grow in adaption phase, centrifuge the culture and exchange the medium without dilution.

* 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.

** If desired, trypsin can also be used. Note: However, the medium must contain a proportion of Serum.

Note: Cells can easily detach due to the reduction of the FBS, so handle with care.

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 adherent culture at 200×g for 5 minutes.
3) Resuspend cells in Xell medium (if necessary, include 6-8 mM L-glutamine) and 1-2 % fetal bovine serum (FBS).
4) Passage cells or change medium by centrifugation every three to four days depending on cell density.
5) Reduce serum concentration while monitoring cell culture viability.
6) Continue cultures until viabilities stabilize at > 90 %.

3.3.4 Freezing of cells

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

1) Choose a well-growing culture with viabilities above 90 %.
2) Prepare a freezing medium consisting of 90 % adHEK 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 with the selected number of cells per 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.

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