Customized solutions for cell cultures.

Protocol for use
Vero GM
Order No. 1030

Contents

1. Product description

Components and specifications

Vero GM 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

Vero GM is a complete chemically-defined, animal-component-free medium. Vero GM is a Xell proprietary medium formulation suitable for cultivation of Vero cells, with a special focus on transfection applications and virus production. The medium is especially designed for transient transfection with e.g. polycationic transfection reagents such as polyethylenimine (PEI). Vero GM supports cell growth and production of e.g. recombinant proteins, viruses 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.

Vero GM 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_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 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 Vero GM.
3) Centrifuge cell suspension at 115×g for 5 minutes.
4) Aspirate supernatant completely and discard.
5) Resuspend the cells in 10 mL Vero GM 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 3–6×10⁵ 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

If you need tips or have any questions on how to cultivate your Vero cells, please contact us.

3.3.3 Freezing of cells

Cells can be frozen in Vero 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 % Vero GM 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.

For further information or assistance contact us.

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