

Technical Bulletin

Frequently Asked Questions: EX-CELL™ Vero Serum-Free Medium

What seeding density should I use for EX-CELL™ Vero and how often should I passage my cells?

Cells should be subcultured using a minimum seeding density of 2×10^4 cells/cm² and subcultured every 3 - 4 days.

What carbon dioxide level do I use with EX-CELL™ Vero?

Incubate flasks in a humidified incubator at 37 C with 5% CO₂.

Does anything need to be added to the medium prior to use?

Yes, prior to use EX-CELL™ Vero liquid medium (Catalog No. 14585) should be supplemented with 4 mM L-glutamine by adding 20 mL/L of a 200 mM solution (Catalog No. 59202).

What cell densities should I expect in T-flasks?

The average cell density achieved during a 4-day passage is approximately 2.5×10^5 cells/cm² in T-flasks.

What is the average doubling time of Vero cells in EX-CELL™ Vero?

The average doubling time in T-flasks is approximately 29 hours \pm 1 hour.

Will the medium support adherent Vero cell culture?

Yes, EX-CELL™ Vero is designed for serum-free growth of Vero cells in adherent conditions, i.e. T-flasks, spinner flasks and bioreactors with microcarriers, etc.

How do I adapt my Vero cells that are currently growing as adherent cultures (in serum-containing medium)?

Vero cells that have been grown in conventional serum-supplemented medium can be readily grown in EX-CELL™ Vero with little or no adaptation. Adaptation to EX-CELL™ Vero requires healthy, viable cultures in mid-logarithmic growth phase. During adaptation, growth rates may be somewhat slower than normal expected rates.

1. Subculture the cells from serum-supplemented medium to EX-CELL™ Vero supplemented with 4 mM L-glutamine using standard trypsinization techniques when cultures reach 100% confluence.
2. Inactivate the trypsin with trypsin inhibitor from Glycine max (soybean) (STI) (0.1%) (Sigma-Aldrich Co., Product No. T6522) at a 1:4-5 relationship of trypsin to STI. Using low-speed centrifugation, pellet the cell suspension at 200 g for 5 minutes and carefully decant the supernatant without disturbing the cell pellet.
3. Resuspend the cells in EX-CELL™ Vero medium supplemented with 4 mM L-glutamine at a density of $2-4 \times 10^4$ cells/cm².
4. Allow the cells to adapt to EX-CELL™ Vero for an additional 4 - 6 passages. Cells are considered fully adapted to EX-CELL™ Vero when growth rates return to normal densities and viabilities are above 95%.
5. Continue to subculture the cells in EX-CELL™ Vero at a density of at least 2×10^4 cells/cm².

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How do I freeze my cells in EX-CELL™ Vero?

Freezing:

Cells can be frozen in EX-CELL™ Vero without the reintroduction of serum.

1. Choose cultures in logarithmic growth with viabilities above 90%.
2. Prepare a freezing medium consisting of 45% cold EX-CELL™ Vero medium, 45% spent medium and 10% dimethyl sulfoxide (DMSO).
3. Using trypsinization protocol as detailed previously, collect and centrifuge the cells at 200 *g* for 5 minutes. Remove the supernatant without disturbing the cell pellet.
4. Resuspend the cells in the freezing medium at 1×10^7 cells/mL.
5. Rapidly transfer 1 - 2 mL of this suspension to sterile cryovials.
6. Place the vials at -20 C for 3 - 4 hours, then transfer to -70 C for 16 - 24 hours.
7. For long-term storage, transfer the vials to liquid nitrogen vapor.

Thawing:

1. Rapidly thaw a vial of frozen cells in a 37 C water bath.
2. Transfer the cells aseptically to a centrifuge tube containing 10 mL of cold EX-CELL™ Vero medium.
3. Using low-speed centrifugation, pellet the cell suspension 200 *g* for 5 minutes and carefully decant the supernatant without disturbing the cell pellet.
4. Resuspend the cells in 5 mL of EX-CELL™ Vero medium.
5. Count the cells for viability and transfer to a sterile tissue culture flask at a seeding density of $2-4 \times 10^4$ cells/cm².
6. Pass the cells using standard cell culture techniques.

What MOI (Multiplicity of Infection) should I use for viral infection?

As there is a great variability dependent on viruses and experimental procedures, it is recommended that you optimize infection conditions for your particular virus. In-house studies performed with Herpes Simplex Type 2 yielded viral titers similar to serum-containing cultures when cultures were infected at a MOI of .01.

What is the glucose level in EX-CELL™ Vero?

EX-CELL™ Vero contains 6 g/L of glucose.

What is the overall protein level in EX-CELL™ Vero? What are the molecular weights of the protein in the medium?

EX-CELL™ Vero contains 0.5 mg/L of protein. All proteins are < 10 kDa.

Are there any animal-derived components in the medium?

No, EX-CELL™ Vero contains no animal-derived components.

Does EX-CELL™ Vero contain a hydrolysate?

Yes, EX-CELL™ Vero contains a hydrolysate, which is plant derived (soybean).

How should I store EX-CELL™ Vero? Can I freeze EX-CELL™ Vero?

The medium should be stored at 2 to 8 C, protected from light. Prolonged exposure to elevated temperatures and/or light may decrease the stability of the product. Freezing of EX-CELL™ Vero is not recommended due to the likelihood of components precipitating upon thawing.

Does EX-CELL™ Vero contain phenol red?

No.

Does EX-CELL™ Vero contain Pluronic® F68?

No.

What is the buffering systems used in EX-CELL™ Vero ?

EX-CELL™ Vero contains sodium bicarbonate to maintain pH. It does not contain HEPES.

For more information about this subject or other SAFC Biosciences' products and services, please call our Technical Services department.

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