SAFC Biosciences™
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Technical Bulletin

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

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

Cells should be subcultured using a minimum seeding density of 3 x 10^5 cells/mL and subcultured every 3 - 4 days.

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

Incubate flasks in a humidified incubator at 37 C with 5% $C0_2$.

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

Yes, prior to use EX-CELL™ HeLa liquid medium (Catalog No. 14591) should be supplemented with 6 mM L-glutamine by adding 30 mL/L of a 200 mM solution (Catalog No. 59202).

What cell densities should I expect in shaker flasks?

The average cell density achieved during a 3-day passage is approximately 2.9 x 10^6 ± 1 x 10^6 cells/mL in shaker flasks.

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

The average doubling time in shaker flasks is approximately 24 ± 6 hours.

Will the medium support adherent HeLa cell culture?

No, EX-CELL™ HeLa is designed for serum-free growth of HeLa cells only in suspension, i.e. shaker flasks, spinner flasks, etc.

How do I adapt my HeLa cells that are currently growing in suspension (either in serum-containing or serum-free medium)?

Cells growing in suspension may be subcultured directly into EX-CELL™ HeLa and adapted using the following procedure. Adaptation to EX-CELL™ HeLa requires healthy, viable cultures in mid-logarithmic growth phase.

1. Subculture the cells from serum-supplemented (or serum-free) medium to EX-CELL™ HeLa supplemented with 6 mM L-glutamine at a minimum seeding density of 3 x 10^5 cells/mL in shaker flasks.
2. Incubate the flasks at 37 C in a humidified incubator with 5% CO_2. Maintain the orbital shaker speed between 120 - 150 rpm.
3. Continue to subculture cells in EX-CELL™ HeLa every 3 - 4 days, using the above seeding density.
4. Allow the cells to adapt to EX-CELL™ HeLa for an additional 4 - 6 passages. Cells are considered fully adapted to EX-CELL™ HeLa when growth rates return to normal and viabilities are above 95%.

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

To adapt adherent cultures to EX-CELL™ HeLa, it is recommended that you gradually wean the cells over a number of passages. As the cells adapt to the serum-free medium, they will lift off the surface and become capable of suspension culture.

1. Using your normal seeding density and trypsinization procedures, subculture the cells into a mixture of medium containing 75% serum-containing medium and 25% EX-CELL™ HeLa. Allow cells to reach normal confluency.
2. At the next subculture, passage the cells into a mixture of medium containing 50% serum-containing medium and 50% EX-CELL™ HeLa. Allow cells to reach normal confluency.

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3. At the next subculture, passage the cells into a mixture of medium containing 25% serum-containing medium and 75% EX-CELL™ HeLa. At this point, most cultures will have lifted off the plate and will be capable of growth in shaker flasks.
4. At the next subculture, passage the cells into 100% EX-CELL™ HeLa. Continue to subculture in EX-CELL™ HeLa until the cells are fully adapted.

Will I experience cell clumping in EX-CELL™ HeLa?

HeLa cultures in EX-CELL™ HeLa exhibit very little clumping. However, it is normal to see aggregates of cells (up to about 40 cells) as the cell density increases (> 3 x 10^6 cells/mL).

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

HeLa cells may be frozen in EX-CELL™ HeLa without the reintroduction of serum or Bovine Serum Albumin (BSA). However, it is necessary to handle the cells gently and freeze the cells under carefully controlled conditions. The addition of D-sucrose to the freezing medium is recommended at a final concentration of 0.1%. Prepare a 10% (100X) solution of D-sucrose in high-quality water and filter through a sterile 0.22 µm membrane filter.

1. Choose cultures in logarithmic growth with viabilities above 90%.
2. Prepare a freezing medium consisting of 45% cold EX-CELL™ HeLa media, 45% spent media, 9.9% dimethyl sulfoxide (DMSO) and 0.1% D-sucrose.
3. Centrifuge the cells at 200 *g* for 5 minutes. Remove the supernatant and prepare the freezing medium.
4. Resuspend the cells in the freezing medium at 1 x 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™ HeLa media.
3. Using low-speed centrifugation, pellet the cell suspension at 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™ HeLa medium.
5. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of 3 x 10^5 cells/mL.
6. Pass the cells using standard cell culture techniques.

What cell density and 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 with Adenovirus (Wild Type Ad5) yielded viral titers similar to serum-containing cultures when cultures were infected at 3 x 10^5 cells/mL at a MOI of 1. Cultures were infected immediately after seeding and optimal harvest time was 48 - 72 hours post-infection.

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

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

What is the overall protein content in EX-CELL HeLa? What are the molecular weights of the protein in the medium?

EX-CELL™ HeLa contains 1.1 mg/L of protein. All proteins are < 10 kDa.

Are there animal-derived components in the medium?

Yes, EX-CELL™ HeLa contains animal-derived components that are considered regulatory friendly.

Does EX-CELL™ HeLa contain a hydrolysate?

Yes, EX-CELL™ HeLa contains a hydrolysate, which is plant derived.

Does EX-CELL™ HeLa contain any surfactants?

EX-CELL™ HeLa contains 0.1% Pluronic® F-68, which is used to minimize cell damage caused by shear forces experienced in suspension culture.

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

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

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

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