

Technical Bulletin

EX-CELL™ 293 Serum-Free Medium for HEK 293 Cells Frequently Asked Questions

What Quality Control testing is performed for each lot?

Test	Method	Product Characteristics	
		Catalog No. 14571	Catalog No. 24571
Appearance	Visual Inspection	Clear yellow solution	Off-white to light beige free-flowing powder
pH	Potentiometric	7.0 - 7.4	For information only
Osmolality	Freezing-point depression	260 - 300 mOsm/kg H ₂ O	For information only
Endotoxin	Kinetic LAL	For information only	For information only
Sterility	Membrane filtration (USP)	No microbial growth detected	N/A
Bioburden	Membrane filtration	N/A	For information only
Growth Promotion	Cell density determination	Minimum cell density of 2 - 4 x 10 ⁶ cells/mL with HEK 293 cells	Minimum cell density of 2 - 4 x 10 ⁶ cells/mL with HEK 293 cells

Is EX-CELL™ 293 manufactured under cGMP conditions?

Yes, all lots of EX-CELL™ 293, Catalog No. 14571 and 24571, are manufactured with strict adherence to cGMP guidelines.

What are the recommended storage conditions?

Medium should be stored at 2 to 8 C, protected from light.

What length of time can the media be out of refrigeration?

Prolonged exposure to elevated temperatures and/or light may decrease the stability of the product. Therefore, conditions other than those recommended should be limited.

SAFC Biosciences ships some products under conditions that are different than the recommended long-term storage conditions. These methods have been shown to not adversely affect the quality or performance of the product.

What is the stability of EX-CELL™ 293 when stored as recommended?

Long-term stability studies are ongoing, however based on our experience with a variety of complex serum-free

formulations, the liquid format is expected to be stable for at least 12 months when stored as recommended. EX-CELL™ 293 dry powder medium has an expected shelf-life of 24 months.

Can I freeze the medium for long-term storage?

As for any cell culture medium, freezing of EX-CELL™ 293 is not recommended due to the risk of component precipitation during thawing.

What is the overall protein content of EX-CELL™ 293?

EX-CELL™ 293 contains approximately 1.1 mg/L of protein.

What are the molecular weights of the proteins in the medium?

All proteins are <10 kDa.

What is the glucose level?

EX-CELL™ 293 contains 6.0 g/L of glucose.

What buffering system is used for EX-CELL™ 293?

EX-CELL™ 293 is buffered using bicarbonate systems.

Does EX-CELL™ 293 contain any surfactants?

Yes, it contains 0.1% Pluronic® F68, which is used to minimize cell damage caused by shear forces associated with suspension cultures.

Are there any components that interfere with downstream purification? What are they?

The formulation for EX-CELL™ 293 contains a hydrolysate and Pluronic® F68. Both of these components can interfere with some downstream purification applications. For additional information regarding downstream purification, please contact Technical Services.

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Can EX-CELL™ 293 be customized?

Yes, SAFC Biosciences has more than 30 years of cell culture experience developing, manufacturing and supporting customized formulations.

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

Liquid EX-CELL™ 293 should be supplemented with 6 mM L-glutamine by adding 30 mL/L of a 200 mM solution (Catalog No. 59202). SAFC Biosciences recommends L-glutamine supplementation of the working volume only. Dry powder EX-CELL™ 293 should be supplemented with 1.8 g/L sodium bicarbonate (Catalog No. 90421) or 24 mL/L of sodium bicarbonate solution 7.5% (Catalog No. 59221).

What types of culture systems are compatible with EX-CELL™ 293?

This medium was designed for the growth of HEK 293 cells in suspension. Roller bottles, shaker flasks, spinner flasks and bioreactors can all be used to grow high density cultures with EX-CELL™ 293.

Will the media support adherent and suspension cultures?

EX-CELL™ 293 is designed for suspension cultures; however, adherent cultures can be supported with the addition of 12 mg/L CaCl₂.

What are recommended culture conditions for EX-CELL™ 293 (T-flask, shaker, bioreactor, spinner)?

Once fully adapted, HEK 293 cells should be passed at a seeding density of 4 x 10⁵ cells/mL in shaker or spinner flasks. Seed 30 mL of cell suspension in 125 mL shaker flasks and 60 mL cell suspension in 250 mL shaker flasks. Shaker speeds should be 100 - 120 rpm and spinner speeds should be 60 - 75 rpm. A 5 - 10% CO₂ environment is recommended.

How do I adapt HEK 293 cells to this medium?

HEK 293 cells can be readily transferred from serum-supplemented medium to EX-CELL™ 293 with little or no adaptation. If adapting from another serum-free medium, we recommend cultures be adapted to Dulbecco's Modified Eagle's Medium/High Modified (DMEM/High) (Catalog No. 51444) supplemented with 6 mM L-glutamine and 5% gamma irradiated Fetal Bovine Serum (FBS) (Catalog No. 12107) for at least three passages prior to adapting the HEK 293 cells to EX-CELL™ 293. The adherent cells in DMEM/High with serum can be detached by trypsin. Inactivate the trypsin with media containing 5% FBS.

1. Subculture the cells from serum-supplemented medium to EX-CELL™ 293 supplemented with 6 mM L-glutamine using standard trypsinization techniques when cultures reach 100% confluence.

2. Inactivate the trypsin with media containing 5% FBS. 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™ 293 media, supplemented with 6 mM L-glutamine at a density of 6 x 10⁵ cells/mL in shaker flasks.
4. Allow the cells to adapt to EX-CELL™ 293 for an additional 4 - 6 passages. Cells are considered fully adapted to EX-CELL™ 293 when growth rates return to normal densities and viabilities are above 95%.
5. Continue to subculture cells in EX-CELL™ 293 at a density of at least 4 x 10⁵ cells/mL into shaker or spinner flasks.

How is viability affected during adaptation? (Is it normal to see a drop in viability during adaptation?)

Adaptation to EX-CELL™ 293 requires healthy, viable cultures in mid-logarithmic growth phase. During adaptation, growth rates may be slower than normal. It is normal to see a drop in viability and a lag of growth during the first two passages in the medium. Usually, 5 - 6 passages in EX-CELL™ 293 are required before your cells are fully adapted.

Will I experience cell clumping during the adaptation period?

HEK 293 cells adapted to EX-CELL™ 293 exhibit very little or no clumping during the first 3 - 5 passages of the adaptation process.

What carbon dioxide levels do I use with EX-CELL™ 293?

Set incubator CO₂ levels to 5 - 10% for best results.

What is the recommended dissolved oxygen level for bioreactor systems?

Adjust dO₂ levels to 50% saturation (calibrated against air).

What is the recommended pH for bioreactor systems?

HEK 293 cells grow best in EX-CELL™ 293 at a pH of 7.0 - 7.2.

What viral assay was used to determine productivity?

TCID₅₀ on HEK 293 cells in DMEM/High + 5% FBS, using Qbiogene's recommended procedure.

What cell density do I use for viral infection? Multiplicity of Infection (MOI)? Harvest time?

For viral production, the following parameters yielded optimal productivity:

- Seeding density – 5 x 10⁵ cells/mL
- Infection – 48 hours post-seeding, with a MOI of 5
- Harvest time – 48 hours post-infection

Is serum or Bovine Serum Albumin (BSA) supplementation necessary for cryopreservation in EX-CELL™ 293?

No, these additives are not necessary. Cells can be frozen down in a freezing medium composed of 45% fresh EX-CELL™ 293, 45% conditioned medium and 10% dimethyl sulfoxide (DMSO).

What types of packaging and volumes are available?

Standard packaging for liquid media is 500 mL and 1000 mL volumes in plastic bottles. A wide variety of containers, including carboys and flexible bags, are available on a custom basis in volumes ranging from 1 to 500 L. Riggings and connectors are chosen based on application needs and specifications.

Standard packaging for dry powder media includes: 1 L, 5 L, 10 L, 50 L and 100 L configurations. For more information, please contact SAFC Biosciences.

Are there animal components in the medium?

Yes, EX-CELL™ 293 contains animal-derived components that are considered regulatory friendly.

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

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