

# Technical Bulletin

## EX-CELL™ 420 Frequently Asked Questions

### How does EX-CELL™ 420 differ from EX-CELL™ 405?

EX-CELL™ 405 is quite different in terms of the lipids, sugars, hydrolysate concentration, amino acids and vitamins.

### Can I use EX-CELL 420 for Sf9, Sf21, Tn5 (High Five™) or all three?

EX-CELL™ 420 can be used for Sf9, Sf21 and Tn5 (High Five™) cells. We recommend supplementing the medium with 4.0 g/L L-glutamine and 1.1688 g/L sodium chloride for optimal Tn5 (High Five™) growth.

### Is EX-CELL™ 420 only for suspension cultures?

No, EX-CELL™ 420 supports the growth of both adherent and suspension cultures.

### How do I go from Grace's Insect Medium + 10% Fetal Bovine Serum (FBS) into EX-CELL™ 420?

Cells can be transferred directly from a serum-containing medium, such as Grace's, into EX-CELL™ 420 without weaning. Cells can also be transferred directly from other serum-free media into EX-CELL™ 420.

### Should I adapt my cells to EX-CELL™ 420 first and then go from stationary to suspension culture, or adapt to suspension and then switch to EX-CELL™ 420?

Cells can be transferred directly from adherent cultures grown in serum-containing or serum-free media into EX-CELL™ 420 suspension cultures without weaning or adaptation.

### Can I go directly from EX-CELL™ 405 into EX-CELL™ 420?

Yes, no adaptation is required. Transfer cells at the normal seeding density.

### Can I use serum with EX-CELL™ 420?

Yes, while the medium is designed for use without serum, serum will not interfere with the performance of EX-CELL™ 420.

### How do I freeze cells using EX-CELL™ 420?

Choose cultures in logarithmic growth with viabilities above 90%. Prepare a freezing medium consisting of 45% cold EX-CELL™ 420 medium, 45% spent medium and 10% dimethyl sulfoxide (DMSO). Centrifuge the cells at 200 *g* for 5 minutes. Remove the supernatant. Resuspend the cells in the freezing medium at 5 x 10<sup>6</sup> to 1 x 10<sup>7</sup> cells/mL. Rapidly transfer 1 - 2 mL of this suspension to sterile cryovials. Place the vials at -20 C for 3 - 4 hours, then transfer to -70 C for 16 - 24 hours. For long-term storage, transfer the vials to liquid nitrogen vapor.

### How do I thaw cells in EX-CELL™ 420?

Rapidly thaw a vial of frozen cells in a 37 C water bath. Transfer the cells aseptically to a centrifuge tube containing 10 mL of cold EX-CELL™ 420 medium. Using low-speed centrifugation, pellet the cell suspension at 200 *g* for 5 minutes and carefully decant the supernatant without disturbing the cell pellet. Resuspend the cells in 5 mL of EX-CELL™ 420 medium. Count the cells for viability and transfer to a sterile shaker flask at a seeding density of 2-4 x 10<sup>5</sup> cells/mL. When the culture has reached a density of 1 x 10<sup>6</sup> cells/mL, passage the cells using standard cell culture techniques.

### Can I freeze EX-CELL™ 420?

No.

### What animal sourced components are in EX-CELL™ 420?

EX-CELL™ 420 contains cod liver oil that is derived by extraction and chemical synthesis from the liver of cod fish. Cod fish have no known risk of Transmissible Spongiform Encephalopathy (TSE) and the extracted lipid contains no animal proteins.

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**Does EX-CELL™ 420 contain lipids (fatty acids)?**

Yes.

**Does EX-CELL™ 420 contain cholesterol?**

Yes.

**Does EX-CELL™ 420 contain antibiotics?**

No.

**Does EX-CELL™ 420 contain methionine?**

Catalog Nos. 14420 and 24420 are formulated with L-methionine while Catalog Nos. 14419 and 14421 are formulated without the addition of L-methionine. However, the yeastolate used in the formulation can contribute low levels of L-methionine.

**What is the ionic strength?**

100 mM.

**What is the glutamine concentration?**

1.000 g/L for Catalog Nos. 14420 and 24420. Catalog Nos. 14419 and 14421 are formulated without the addition of L-glutamine.

**Should I supplement the medium with L-glutamine?**

Catalog Nos. 14420 and 24420 contain L-glutamine. For Catalog Nos. 14419 and 14421, add 1.000 g/L. If you are using the product to grow Tn5 (High Five™) cells we recommend adding 4.0 g/L L-glutamine.

**What is the buffering system?**

EX-CELL™ 420 uses a phosphate buffering system.

**What is the phosphate concentration?**

8.4 mM.

**Does EX-CELL™ 420 contain a surfactant? Which one?**

**What is the concentration?**

EX-CELL™ 420 contains Pluronic® F68 at a concentration of 0.1% to minimize cell damage caused by shear forces and and/or bubble ruptures in suspension cultures.

**What is yeast hydrolysate?**

Yeast extract hydrolyzed (digested) by plant enzymes. This digest is then passed through a 10,000 MW cutoff filter and freeze-dried.

**Is it compatible with ammonium sulfate precipitation and nickel columns (HIS-Tag)?**

Generally, we recommend dialysis with a 10,000 MW cutoff to remove the Pluronic® F68, which may interfere with purification.

**Which medium will give a higher expression?**

It depends on the cell line and the protein of interest.

**Can it be autoclaved?**

No.

**What is the iron concentration?**

Approximately 2.0 µM.

**What is the zinc concentration?**

The formulation does not contain zinc, but zinc is provided by the yeast hydrolysate.

**What is the calcium concentration?**

4.5 mM.

**Do you have a phosphate-free formulation?**

No, but this medium can be made as a custom.

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

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