

# EX-CELL® Advanced CHO Fed-batch Medium

## Chemically Defined Fed-batch Medium

### Product description

EX-CELL® Advanced CHO Fed-Batch Medium is chemically defined, animal component free, and contains no hydrolysates or components of unknown composition. The formulations was developed using multivariate analysis of 10,000+ data points that included performance, physical, regulatory and safety design specifications.

This medium supports high growth and viability and is designed for use in Fed-batch applications in conjunction with EX-CELL® Advanced Feed 1 supporting superior performance on all common industrial Chinese Hamster Ovary (CHO) and CHOZN® cell lines.

For alternative feeding a combination with Cellvento® 4 Feed can be recommended to boost performance.

The medium is available in liquid and dry powder format.

### Product Handling & Storage

Do not use if liquid medium is cloudy or contains precipitates. Powder product should be stored dry at 2–8 °C and protected from light. Liquid product should be stored at 2–8 °C and protected from light for up to one year. Use aseptic technique when handling or supplementing this medium. Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

### Reconstitution Method

#### Preparation Instructions for Powder, Cat. No. 24366C

1. Measure out 80% of final required volume of Milli-Q® or similar purified water intended for cell culture use. Recommended water temperature is 25–40 °C. While stirring, slowly add the powder medium at 22.09 g/L.
2. Continually stir for 15 minutes. Product will remain slightly turbid.

3. Adjust pH to 5.0 using NaOH.
4. Add 1.9 g/L of Sodium Bicarbonate.
5. Continually stir for at least 30 minutes until product is clear.
6. Adjust the pH to 7.2+/- 0.1 using NaOH or HCl.
7. QS to final volume.
8. Measure osmolality. Product should be 280–320 mOsm/kg.
9. Immediately filter using a sterilising-grade filter ( $\leq 0.22 \mu\text{m}$ ). For recommendations, see Page 2.
10. Store product at 2–8 °C in the dark until use.

EX-CELL® Advanced CHO Fed-Batch Medium is formulated without L-glutamine and without hypoxanthine/thymidine. Aseptic supplementation instructions:

1. Add L-glutamine (**Cat.No. 100286**), 2–8 mM final concentration, before use in applications not requiring GS selection.
2. Add HT Media Supplement (**Cat. No. H0137**) before use in applications not requiring DHFR selection.

Note: Shelf life of the product may be affected by the nature of the supplements.

### Initiating Cultures

1. Rapidly thaw (<1 minute) a vial of frozen cells in a 37 °C water bath.
2. Transfer the entire contents aseptically into a 125-mL shake flask containing 30 mL prewarmed complete EX-CELL® Advanced CHO Fed-Batch Medium.
3. Incubate at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> in air on an orbital shaker platform (19 mm diameter orbit) rotating at 120–140 rpm.
4. Maintain cell density between 0.5–1 ×10<sup>6</sup> viable cells/mL for the first two passages following recovery; thereafter, return to your normal maintenance schedule.

## Subculturing

1. Verify that the incubator is set to 37 °C, 5% CO<sub>2</sub>, and has water for humidity control (~80%).
2. Pre-warm complete EX-CELL® Advanced CHO Fed-Batch Medium to room temperature.
3. Aseptically remove a small volume of cell culture sample from the flask and count by trypan blue exclusion using a hemocytometer or an automated cell counter. Do not proceed if cell viability is less than 90%. Note: If cell viability is below 90%, troubleshoot conditions prior to continuing.
4. Determine the correct volume of cell culture to inoculate a new flask at a starting cell density of 2–3 x10<sup>5</sup> cells/mL in a total volume of 30 mL fresh EX-CELL® Advanced CHO Fed-Batch Medium per 125-mL shake flask.
5. Aseptically transfer the appropriate amount of cells to the new flask, and add pre-warmed medium up to the desired volume.
6. Incubate flasks in a humidified 37 °C incubator with 5% CO<sub>2</sub> on an orbital shaker at 120–140 rpm.
7. Passage cells by repeating the above steps at least twice weekly, and expand culture volume as necessary. Note: When passing the cells, medium carryover should not exceed 25% of the final volume. If carryover exceeds 25%, centrifugation is recommended.

## Cryopreservation

1. Prepare the desired quantity of cells, harvesting in mid-logarithmic phase of growth with viabilities >90%.
2. Prepare a freezing medium consisting of 46.5% cold EX-CELL® Advanced CHO Fed-Batch Medium, 46.5% conditioned medium and 7% dimethyl sulfoxide (DMSO).
3. Harvest cells by centrifugation at 200 g for five minutes. Remove the supernatant.
4. Resuspend cell pellet in the freezing medium at 5 x10<sup>6</sup> to 1 x10<sup>7</sup> cells/mL.
5. Rapidly transfer 1–2 mL of this suspension to sterile cryovials.
6. Place the vials in a controlled rate freezing apparatus following standard procedures (1 °C decrease per minute).
7. For long-term storage, transfer the vials to liquid nitrogen (vapor phase).

## To place an order or receive technical assistance

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## Ordering information

### For EX-CELL® Advanced Fed-Batch Medium

Cat. No.	Product name	Pkg. size	Equivalent
14366C-1000ML	EX-CELL® Advanced Fed-Batch Medium	1000 mL	1 liter
24366C-1L	EX-CELL® Advanced Fed-Batch Medium	0.022 kg	1 liter
24366C-10L	EX-CELL® Advanced Fed-Batch Medium	0.221 kg	10 liter
24366C-100L	EX-CELL® Advanced Fed-Batch Medium	2.210 kg	100 liter

### For compatible Feeds

Cat. No.	Product name	Pkg. size
24367C	EX-CELL® Advanced Feed 1 with glucose	1L, 10L, 50L
24368C	EX-CELL® Advanced Feed 1 without glucose	1L, 10L, 50L
103796	Cellvento® 4Feed	1L, 10L, 50L

### For cell culture additives

Cat. No.	Product name	Pkg. size
1.37020.5000	Sodium hydroxide pellets suitable for the biopharmaceutical production EMPROVE® bio	5 kg
1.37013.2500	Sodium hydrogen carbonate suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, USP, JP	2.5 kg
1.00286.1000	L-Glutamine suitable for use as excipient EMPROVE® exp DAB, USP	1 kg

### For EX-CELL® Advanced Fed-Batch Medium

	Bacteria Removal	Mycoplasma & Bacteria Removal	Virus, Mycoplasma & Bacteria
Volume (L)	Millipore® Express SHC	Millipore® Express SHR with Prefilter	Viresolve® Barrier
1	KHGES015FF3	KHVES015FF3	VBKG005TC1
10	KHGES015FF3	KHVES015FF3	VBKG015TC1
100	KHGES006FF3	KHVES03TT3	VBKG100TC1

## How to Order

For additional information, please contact your Regional representative or call Customer Service at or visit our website at [SigmaAldrich.com/CHOperformance](https://www.sigmaaldrich.com/CHOperformance)

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