SAFC®
Pharma & Biopharma Raw Material Solutions

Product Information

# EX-CELL® Advanced HD Perfusion Medium

## Chemically Defined Cell Culture Medium

### Product Description

EX-CELL® Advanced HD Perfusion Medium is a chemically defined cell culture medium designed specifically for intensified perfusion processes with Chinese Hamster Ovary (CHO) cells. This medium was developed to reach and maintain high cell densities at low cell specific perfusion rates (CSPR), while supporting high volumetric productivities of monoclonal antibodies and recombinant proteins in suspension culture. Performance of this medium in perfusion has been validated with different relevant industrial CHO cell lineages (CHOZN® GS, CHO-S, CHO-DG44, CHO-K1) producing a variety of proteins. The formulation is animal-component free and has been formulated without L-Glutamine.

### Application

EX-CELL® Advanced HD Perfusion Medium can be used as amplification medium and production medium in perfusion applications.

This product is intended for research or further manufacturing in the bio-manufacturing industry, but not for human or therapeutic use.

### Reconstitution Method to Prepare EX-CELL® Advanced HD Perfusion Medium

Hydration for this medium should follow the instructions below:

1. Measure 80% of the final required volume of Milli-Q® or similar cell culture grade water at room temperature (15–25 °C) in an appropriately sized container.
2. Slowly add 28.57 g/L of medium, while stirring. Continue stirring for 15 minutes. Product will remain slightly turbid.
3. Measure pH and adjust to 9.1 +/– 0.05 with 5N NaOH. Continue stirring for 10 minutes. Product will remain slightly turbid.
4. Adjust pH to 6.4 ± 0.1 using 5N HCl.
5. Add 1.565 g/L sodium bicarbonate to the solution and stir continuously for 15 minutes.
6. Adjust to pH 7.3 ± 0.1 using 5N NaOH.
7. Add Milli-Q® or similar cell culture grade water to reach 100% final volume.
8. Measure osmolality. Final osmolality should be at 320–360 mOsmol/kg.
9. Immediately filter using a sterilizing-grade (≤0.22 µm) bottle cap or capsule filter.
10. Store product at 2–8 °C protected from light.
    - Reconstituted medium is stable for at least 30 days.

**Note:** This medium does NOT contain L-Glutamine. Aseptic supplement as required prior to use.



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## Storage

- Dry powder should be stored dry at 2–8 °C and protected from light.
- Do not use after expiration date.

## Using EX-CELL® Advanced HD Perfusion Medium in Perfusion Mode

- Add L-Glutamine to EX-CELL® Advanced HD Perfusion Medium prior to use with non-GS CHO cell lines. Recommended concentrations varied depending on the cell line. A starting concentration of 4–6 mM is suggested.
- Supplementation with a surfactant (e.g., poloxamer) is not required to use this product.
- Cell selection agents should be added as required during the seed train expansion.
- This medium contains <2 µM thymidine. While not required, 1× HT supplement can be added prior to use with non-dhfr systems.

## Direct Medium Adaptation

Cell lines may be adapted directly into EX-CELL® Advanced HD Perfusion Medium. Cells should be seeded at 3–5×$10^5$ cells/mL, then subcultured when densities reach 1–2×$10^6$ cells/mL and ≥80% viability. Adaptation is completed when cells attain a stable doubling time and ≥90% viability over at least 2–3 passages.

Cells adapted to EX-CELL® Advanced HD Perfusion Medium can be directly thawed and cultured in EX-CELL® Advanced HD Perfusion Medium.

## Sequential Medium Adaptation

The adaptation guidance provided below relies on regular sub-culturing of cells to maintain cultures in a logarithmic growth phase. This typically means that cells should be passaged every 3 to 4 days. At least two passages at each adaptation step are recommended to ensure that cells appropriately adjust to their new media environment.

## Subculturing

1. Pre-warm hydrated EX-CELL® Advanced HD Perfusion Medium to room temperature.
2. Aseptically remove a small volume of cell culture sample from the flask and count by trypan blue exclusion using a hematocytometer or an automated cell counter. Viability should be higher than 90% at all times.
3. Determine the correct volume of cell culture to inoculate a new flask at a starting cell density of 2–3×$10^5$ viable cells/mL for the targeted working volume.
4. Aseptically add the corresponding amount of fresh media pre-warmed at room temperature to the new flasks followed by the calculated amount of cells.
5. Incubate at 37 °C in a humidified atmosphere of 5% $CO_2$ in air on an orbital shaker platform (19 mm diameter orbit) rotating at 120–140 rpm.
6. Passage cells by repeating the above steps at least twice a week, and always maintaining the cells in exponential growth phase.

**Note:** When passaging cells, medium carry over should not exceed 25% for the final volume. If carryover exceeds 25% centrifugation is recommended.

| Ratio of Current Media vs. EX-CELL® Advanced HD Perfusion Medium (in %) | Seeding Density (×$10^5$ cells/mL) | Evaluation of Cell Growth | Acceptance Criteria for Next Step |
|---|---|---|---|
| 75:25 | 3.0 | Cell density, viability in mid-log growth phase | Normal cell doubling time; Viability >80% over at least 2 passages |
| 50:50 | 3.0 | Cell density, viability in mid-log growth phase | Normal cell doubling time; Viability >80% over at least 2 passages |
| 25:75 | 3.0 | Cell density, viability in mid-log growth phase | Normal cell doubling time; Viability >80% over at least 2 passages |
| 10:90 | 3.0 | Cell density, viability in mid-log growth phase | Normal cell doubling time; Viability >80% over at least 2 passages |
| 0:100 | 3.0 | Cell density, viability in mid-log growth phase | Adaptation complete when cells maintain normal doubling time; Viability ≥90% over at least 2 passages |

## Cryopreservation

Adapted cells to EX-CELL® Advanced HD Perfusion Medium may be created.

1. Prepare the desired quantity of cells, harvesting in mid-logarithmic phase of growth with viabilities over 90%.
2. Prepare a freezing medium consisting of 46.5% cold EX-CELL® Advanced HD Perfusion Medium, 46.5% conditioned medium and 7% dimethyl sulfoxide (DMSO).
3. Harvest cells by centrifugation at 200 xg for 5 minutes and carefully remove the supernatant.
4. Re-suspend cell pellet in the freezing medium at 10–20×$10^6$ viable cells/mL.
5. Rapidly transfer 1–2 mL of this suspension to sterile cryovials.
6. Place the vials in a cryobox or 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 is recommended).

## Thawing

1. In a 50 mL centrifuge tube, prepare 10 mL culture medium under the clean bench or the laminar flow hood.
2. Rapidly thaw (<1 minute) a vial of frozen cells in a 37 °C water bath. Take out of the vial when ice particles detach from the side of the vial (DMSO at high concentration can have a toxic effect at higher temperature).
3. Transfer the content of the vial the conical tube from step 1 and centrifuge it at 200 xg for 5 minutes. After supernatant is discarded, cells can then be re-suspended in 10 mL of medium and transferred to a shake flask containing 20 mL of medium.
   - Alternatively, the entire content of the vial can be transferred aseptically into a 125 mL shake flask containing 28–29 mL pre-warmed complete EX-CELL® Advanced HD Perfusion Medium.
4. Incubate at 37 °C in a humidified atmosphere of 5% $CO_2$ in air on an orbital shaker platform (19 mm diameter orbit) rotating at 120–140 rpm.

## Ordering Information for EX-CELL® Advanced HD Perfusion Medium

| Cat. No. | Product Name | Pkg. Size | Equivalent |
|---|---|---|---|
| **24370C-1L** | EX-CELL® Advanced HD Perfusion Medium | 28.56 g | 1 liter sample |
| **24370C-5L** | EX-CELL® Advanced HD Perfusion Medium | 0.142 kg | 5 liters |
| **24370C-50L** | EX-CELL® Advanced HD Perfusion Medium | 1.428 kg | 50 liters |
| **24370C-200L** | EX-CELL® Advanced HD Perfusion Medium | 5.714 kg | 200 liters |
| **24370C-500L** | EX-CELL® Advanced HD Perfusion Medium | 14.285 kg | 500 liters |
| **24370C-800L** | EX-CELL® Advanced HD Perfusion Medium | 22.856 kg | 800 liters |

## Ordering information 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 |

## Ordering information for aseptic filters

| Cat. No. | Product Name | Pkg. Size |
|---|---|---|
| **GPWP02500** | Millipore Express® PLUS Membrane, 0.22 µm, 25 mm | 100 |
| **GVWP02500** | Durapore® Membrane, 0.22 µm, 25 mm | 100 |

## To place an order or receive technical assistance

In Europe, please call Customer Service:
France: 0825 045 645
Germany: 069 86798021
Italy: 848 845 645
Spain: 901 516 645 Option 1
Switzerland: 0848 645 645
United Kingdom: 0870 900 4645

For other countries across Europe,
please call: +44 (0) 115 943 0840

Or visit: **MerckMillipore.com/offices**

For Technical Service visit:
**MerckMillipore.com/techservice**

**MerckMillipore.com**

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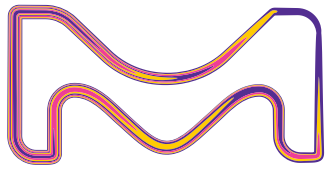



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