High-Intensity Perfusion CHO Medium

Catalog Numbers A4230201, A4230202, A4230203, A4230204

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ High-Intensity Perfusion CHO Medium is a chemically defined, animal origin free, protein free medium. This AGT™ medium is designed to support CHO cell clones in workflows that incorporate perfusion with intent to operate at high cell densities while reducing required medium exchange rates. The medium is formulated without hypoxanthine and thymidine for use in dihydrofolate reductase (DHFR)-amplified systems, without L-glutamine for use in glutamine synthetase systems, and without phenol red to minimize estrogen-like effects of phenol red. The glucose concentration is formulated to minimize potential lactic acid accumulation under typical culture conditions. High-Intensity Perfusion CHO Medium also allows reconstitution at multiple concentrations for adaptation and seed train applications while providing high medium depth to help cells thrive in the most demanding perfusion environment.

Contents and storage

Product	Cat. No.	Amount	Storage	Shelf life[1]
High-Intensity Perfusion CHO Medium	A4230201	2 L	• 2°C to 8°C • Protect from light • Keep dry	12 months
	A4230202	15 L		
	A4230203	100 L		
	A4230204	370 L		

[1] Shelf Life is determined from Date of Manufacture.

Culture conditions

Media: High-Intensity Perfusion CHO Medium (HIP CHO Medium)

Cell line: Chinese Hamster Ovary (CHO)

Culture type: Suspension

Culture vessels: shake flasks, spinner bottles (rpm may vary with shaker platform/impeller design and should be empirically determined for optimal cell growth), or bioreactor.

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 8% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Flexibility in reconstitution

HIP CHO Medium can be flexibly reconstituted following these recommendations:

Concentration	Approx. mOsmo/kg	Require salt addition
1 x (34.92 g/L)	375 +/- 25	No
0.8 x (29.1 g/L)	310 +/- 25	No
0.66 x (23.3 g/L)	248 +/- 25	Yes

Reconstitute HIP CHO Medium – No salt addition

This concentration is intended for use in production perfusion environments to achieve maximum cell density and productivity at minimum medium exchange rates.

For Research Use or Further Manufacturing. Not for diagnostic use or direct administration into humans or animals.

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1. Measure 90% of the final volume of deionized or distilled water at room temperature (15°C to 30°C).
2. Add HIP CHO Medium at 34.92 grams/L to water.
3. Mix for a minimum of 20 minutes.
4. Using a calibrated vessel, dilute to final production volume with ambient deionized or distilled water.
5. Mix for an additional 20 minutes.
6. Measure the pH, then check and record osmolality.
7. Sterilize immediately by membrane filtration (positive pressure recommended).

Reconstitute HIP CHO Medium – With salt addition

This concentration is intended for use to facilitate adaptation and low cell density operations of 310 mOsmo/kg.

1. Measure 90% of the final volume of deionized or distilled water at room temperature (15°C to 30°C).
2. Add HIP CHO Medium at 23.3 grams/L to water.
3. Mix for a minimum of 20 minutes.
4. Using a calibrated vessel, dilute to final production volume with ambient deionized or distilled water.
5. Mix for an additional 20 minutes.
6. Measure the pH, then check and record osmolality (z).
7. Re-adjust the osmolality to 310 mOsmo/kg by filling out the following table and using the formula in the first column.

<table>
<tr><th>Formula</th><th>Target osmolality (x)</th><th>Current osmolality (z)</th><th>Final volume (L)</th><th>additional NaCl (y)</th></tr>
<tr><td rowspan="4">y grams NaCl = ((x - z) / 32) * L</td><td>310 mOsmo/kg</td><td>____</td><td>____</td><td rowspan="4"></td></tr>
<tr><td colspan="2">x - z = ____</td><td></td></tr>
<tr><td colspan="2">(x - z) / 32 = ____</td><td></td></tr>
<tr><td colspan="3">((x - z) / 32) * L = ____</td><td>____</td></tr>
</table>

8. Re-adjust the osmolality from _____mOsm/kg to 310 mOsm/kg using the following calculation. (310-_____mOsm/kg from above) / 32 x____L TPV=_____g NaCl to add. 10.
9. Mix for a minimum of 15 minutes.
10. Sterilize immediately by membrane filtration (positive pressure recommended).

Prepare complete medium

When used in a non glutamine synthetase based system, HIP CHO Medium requires aseptic supplementation with L- glutamine or GlutaMAX™ Supplement prior to use.

1. Add GlutaMAX™ Supplement or L-glutamine at 2–8 mM final concentration to the medium before use.

   When unsure what is optimal for your clone, start with 4 mM.

2. Add Anti-Clumping Agent (1 mL/L) to the medium after transfection to reduce cell aggregation if required.

   Note: Consider reducing L-glutamine concentration if the cell line in use is sensitive to ammonia. Addition of a surfactant such as Pluronic™ F-68 is not required.

Recover cells

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a 125-mL shake flask containing 30 mL of pre-warmed complete HIP CHO Medium.
3. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 125–135 rpm.
4. Maintain a cell density of 0.5 to 1.0 × 10^6 viable cells/mL for the first two passages following recovery; thereafter, return to your normal maintenance schedule.

   Note: Do not centrifuge cells to remove DMSO as they are extremely fragile upon recovery from cryopreservation.

Subculture cells

We recommend thawing a fresh, low-passage vial of cells for use every 3 months or 30 passages.

1. Determine viable cell density using a Countess™ II Automated Cell Counter (alternate automated or manual methods may also be used).
2. Ensure that the cell density is ≥1 × 10^6 viable cells/mL, viability is ≥90%, and the growth rate is in mid-logarithmic phase prior to subculturing.
3. Calculate the volume of cell culture and medium necessary to seed a flask at 2 × 10^5 – 3 × 10^5 viable cells/mL in a total volume of 30 mL of fresh HIP CHO Medium per 125-mL shake flask.

   Note: If cell density does not reach 1 × 10^6 viable cells/mL within 5 days of recovery, centrifuge cells at 100 × *g* for 5 minutes and resuspend the cell pellet in 20–30 mL of fresh complete medium.

4. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 115–135 rpm.
5. For optimal performance and cell growth, dilute the cells at a seeding density of 3 × 10^5 viable cells/mL every 3–4 days with fresh HIP CHO Medium.

Adapt CHO Cells to HIP CHO Medium

We recommend adapting CHO cells to HIP CHO Medium using sequential adaptation. However some CHO cell lines will adapt directly from other serum-free medium. Both methods of adaptation may be run in parallel to help determine this.

Before initiating adaptation procedures, it is critical that:

- Any growth factors or hydrolysates or similar components are identified and supplemented into your complete HIP CHO Medium
- Cell viability be ≥90%
- The growth rate be in mid-logarithmic phase

Note: It is possible that your cell clone may perform well without these additional supplements but that should be determined as a separate action.

Sequential adaptation

Note: During sequential adaptation of CHO cells grown in conventional 5–10% serum-supplemented medium or other serum-free medium, use a seeding density of $3 \times 10^5 – 4 \times 10^5$ viable cells/mL.

1. Monitor cell growth using Countess™Automated Cell Counter until viable cell density reaches $\geq 1 \times 10^6$ cells/mL.

2. Subculture cells into stepwise increasing ratios of HIP CHO Medium to original medium with each subsequent passage (25:75, 50:50, 75:25, 90:10 followed by 100% HIP CHO Medium).

   We recommend maintaining backup cultures in the original ratio medium until success with the new ratio medium is achieved.

   Note: Multiple passages at each ratio may be needed to achieve consistent growth.

After several passages in HIP CHO Medium, the viable cell count should reach at least 2×10^6 cells/mL with ≥85% viability within 3–4 days of seeding the culture. At this stage, the culture is considered to be adapted to HIP CHO Medium.

Direct adaptation

Note: If suboptimal performance is achieved using the direct adaptation method, use the sequential adaptation method.

1. Subculture cells into 100% HIP CHO Medium using a seeding density of $3 \times 10^5 – 4 \times 10^5$ viable cells/mL when subculturing.

2. Continue to subculture cells at $3 \times 10^5 – 4 \times 10^5$ viable cells/mL (every 3–4 days) until consistent growth is achieved.

   When cell growth has been demonstrated, the seeding density may be reduced to $2 \times 10^5 – 3 \times 10^5$ viable cells/mL during the final stages of adaptation.

After several passages in HIP CHO Medium, the viable cell count should reach at least 2×10^6 cells/mL with ≥85% viability within 3–4 days of seeding the culture. At this stage, the culture is considered to be adapted to HIP CHO Medium.

Cryopreserve cells

Prepare the desired quantity of cells, harvesting in mid-log phase of growth with viability >90%.

1. Prepare the required volume of cryopreservation medium of 92.5% HIP CHO Medium (conditioned media) + 7.5% DMSO, and store at 4°C until use.

   IMPORTANT! Prepare cryopreservation medium on the day of use.

2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of 1×10^7 viable cells/mL.

3. Harvest cells by centrifugation at 100 × *g* for 5–10 minutes.

4. Resuspend cell pellet in the pre-determined volume of 4°C of cryopreservation medium.

5. Immediately dispense aliquots of this suspension into cryovials according to the manufacturer's specifications.

6. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).

7. Transfer frozen cells to liquid nitrogen; (vapor phase) storage at –200°C to –125°C.

   Note: Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
L-Glutamine (200 mM)	25030
GlutaMAX™ Supplement (200 mM)	35050061
Anti-Clumping Agent	0010057
Freedom™ CHO-S™ Kit	A1369601
Countess™ II Automated Cell Counter	AMQAX1000

Limited product warranty

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