

HYCLONE MEDIA AND SUPPLEMENTS

HyCell CHO media containing hypoxanthine and thymidine (HT) are production media, while HyCell CHO media without HT are selection system compatible. For a long shelf-life and compatibility with glutamine synthetase (GS) systems, please note that neither of these formulations include L-glutamine. Recommended L-glutamine concentration is 4 to 6 mM. HyCell CHO is available in liquid and powder formats in user-friendly packaging (Fig 1).

- Chemically defined and animal-derived component-free formulations
- Metabolically designed for high cell yield and recombinant protein production
- Allow direct or sequential adaptation
- Designed for large-scale culture applications, including perfusion and fed-batch strategies

- Without L-glutamine
- Without poloxamer 188
- Without sodium bicarbonate or HEPES
- Without phenol red

- With sodium bicarbonate
- With poloxamer 188
- Without phenol red
- Without L-glutamine
- Without HEPES



An optimal cell culture process is dependent of a variety of factors including the parental cell line, the genetic makeup of the specific clone, medium and feed composition, as well as process variables to maximize viable cell densities and titers while maintaining cell morphology. Our experts in medium design and development know and understand how these factors can influence the metabolic processes involved. They evaluate the culture's metabolic activities, measuring nutritional demand and waste creation to make sure the correct type and quantity of nutrients are used to minimize waste and resultant cell toxicity. Our experts use their understanding of metabolic pathways to optimize medium composition for enhanced productivity and viable cell densities. Once a medium has been optimized using this Metabolic Pathway Design process, our scientists can help you devise the most effective cell culture strategy using a combination of medium and feeds to further enrich productivity and reduce process inefficiencies.

Product handling

- Both the dry powder medium (DPM) and hydrated medium should be stored at 2°C to 8°C, away from light
- If culturing in 5% CO₂ environment, add sodium bicarbonate to a concentration of 2.2 g/L. If culturing in 10% CO₂ environment, add sodium bicarbonate to a concentration of 3.6 g/L
- Additional buffering can be achieved by adding HEPES (1 to 2 g/L)
- Supplement with 1.0 g/L poloxamer 188. In high-shear environments, supplement with up to 1.4 g/L poloxamer 188

Protocol for hydration of HyCell CHO powder medium

- While stirring, add 25.40 g/L HyCell CHO powder medium with HT (SH30933) or 25.39 g/L HyCell CHO powder medium without HT (SH30948) to cell culture grade water at 90% of final preparation volume. Mix until dissolved. Using warmer water will improve solubility. If your water source is normally cool, it may be useful to adjust the water temperature before adding DPM.
- Add poloxamer 188, sodium bicarbonate, and HEPES and mix until dissolved.
- Bring vessel to final volume with cell culture grade water. Allow solution to mix for 20 min.
- Check pH and osmolality and adjust if necessary. Expected values: pH 7.0–7.4, osmolality 280–320 mOsm/kg.
- Sterile filter into desired container using a 0.2 µm sterile filter.

Preparation notes

L-glutamine can be added (4–6 mM final concentration) during step 2 if medium is to be stored short term. For longer storage, it is recommended that L-glutamine is added at time of use. Once hydrated, non-glutamine containing medium can be stored at 2°C to 8°C for 12 months.

Instructions for use for HyCell CHO liquid medium

With the exception of glutamine addition, HyCell CHO liquid medium is ready to use right out of the bottle. Recommended addition is 4–6 mM sterile filtered L-glutamine.

General culture recommendations

- Cultures should be incubated at 37°C in a 5% CO₂ environment.
- Maintain adapted cells by establishing mid-logarithmic growth phase subculturing schedule.
- Suggested seeding density of cultures: 2.5 × 10⁵ cells/mL; viability should be > 90%.

Direct adaptation

Transfer cells grown in current serum-free medium directly into HyCell CHO at 3.0 × 10⁵ cells/mL. Passage cells every 3 to 4 day. Adaptation is complete once cells have transitioned to a growth rate of ~ 24 h per doubling.

Sequential adaptation

Sequential adaptation can be accomplished by beginning with a 50/50 mixture of existing medium/test medium and culturing as above, reducing the concentration of the existing medium by 50% with each passage. Generally cells will adapt within 3 to 4 passages.

Cryopreservation

Adapted cells can be cryopreserved in HyCell CHO with 10% DMSO. We recommend freezing the cells at a minimum cell density of 1 × 10⁷ cells/mL.

Quality control testing

Quality control test specifications are listed in Table 1.

Table 1. Test specifications¹

Appearance	Clear yellow solution
Osmolality	270 to 330 mOsm/kg
pH	7.0 to 7.4
Sterility	No growth (bacteria or fungi)
Endotoxin	< 10.0 EU/mL ¹
Application	Growth promotion ¹

¹ Refer to certificate of analysis for actual results.

Custom production

Formulations and delivery systems can be customized to your specific process requirements or optimized to maximize process yields.

Rapid Response Production (RRP)

Our RRP program manufactures up to 200 L of your custom prototype formulation within seven working days of your request. Use our RRP service to expedite the development and testing of custom buffers and process liquids for your biopharmaceutical manufacturing process.

Related products

HyClone Cell Boost kit

Cell Boost™ Process Supplements (100 g each) contain samples of supplements designed to increase cell productivity in a variety of cell lines (Table 2). Each supplement is developed through the Metabolic Pathway Design process and is chemically-defined and protein-free with no animal derived components.

Table 2. Supplement matrix

	Amino acids	Vitamins	Glucose	Trace elements	Growth factors	Hypoxanthine/thymidine	ADCF* lipids	ADCF* cholesterol	Suitable for	Code number
Cell Boost 1 Supplement (R05.2)	•	•	•						HEK293 CHO	SH30584
Cell Boost 2 Supplement (R15.4)	•		•						PER.C6™ CHO	SH30596
Cell Boost 3 Supplement (JM3.5)	•	•	•	•		•			Hybridoma Myeloma	SH30825
Cell Boost 4 Supplement (PS307)	•	•	•	•	•		•	•	CHO	SH30857
Cell Boost 5 Supplement (CN-F)	•	•	•	•	•	•	•	•	Hybridoma NS0 HEK293 CHO	SH30865
Cell Boost 6 Supplement (CN-T)	•	•	•	•	•	•	•	•	T-Cells Hybridoma NS0 HEK293 CHO	SH30866

* Animal-derived component-free

Ordering information

HyCell CHO medium is manufactured in homogenous liquid lot sizes up to 10 000 L and powder lots up to 250 000 L.

Product	Size	Code number
HyClone HyCell CHO powder medium	10 L bottle	SH30933.02*
With HT	50 L bottle	SH30933.03*
Without L-glutamine	100 L polybag/pail	SH30933.04*
Without poloxamer 188	500 L polybag/pail	SH30933.05*
	1000 L polybag/drum	SH30933.06*
HyClone HyCell CHO liquid medium	1 L bottle	SH30934.01*
With HT	5 L bag	SH30934.03†
With sodium bicarbonate	10 L bag	SH30934.04†
With poloxamer 188	20 L bag	SH30934.05†
Without L-glutamine	50 L bag	SH30934.06†
	100 L bag	SH30934.07†
	200 L bag	SH30934.08†
HyClone HyCell CHO powder medium	10 L bottle	SH30948.02*
Without HT	50 L bottle	SH30948.03*
Without L-glutamine	100 L polybag/pail	SH30948.04*
Without poloxamer 188	500 L polybag/pail	SH30948.05*
	1000 L polybag/pail	SH30948.06*
HyClone HyCell CHO liquid medium	1 L bottle	SH30949.02*
Without HT		
With sodium bicarbonate		
With poloxamer 188		
Without L-glutamine		
L-glutamine 200 mM	100 mL bottle	SH30034.01*
	500 mL bottle	SH30034.02*
	500 g	SH30336.03†
HyClone Cell Boost kit	6 × 100 g	SH30890*

* Item in stock.

† Item is made to order. Lead times and minimum order quantities apply.

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