

# SFM4CHO

## HYCLONE MEDIA AND SUPPLEMENTS

HyClone™ SFM4CHO is a serum-free and protein-free cell culture medium designed for high performance in a variety of culture applications. The medium supports the dihydrofolate reductase (DHFR) selection/amplification system and is available with and without L-glutamine to support the glutamine synthetase (GS) gene expression system. SFM4CHO is available in user-friendly packaging in liquid and powder format (Fig 1).

Key features of SFM4CHO medium include

- Protein-free formulation
- Designed for high cell yield and recombinant protein production
- Allows for direct or sequential adaptation
- Designed for large-scale culture applications, including perfusion and fed-batch strategies
- Manufactured according to cGMP guidelines

## Specifications

- Protein-free
- Contains poloxamer 188
- Does not contain phenol red
- Available with or without L-glutamine

### Product handling

Store medium at 2°C to 8°C, away from light. In addition, powder medium should be stored protected from moisture in a tightly sealed container.

## Suggested preparation

### Reconstitution of SFM4CHO powder medium

1. While stirring, add SFM4CHO dry powder to cell culture-grade water at 90% of final preparation volume (19.83 g/L). If your water source is normally cool, it can be useful to adjust the water temperature. Using warmer room temperature water (22°C to 25°C) will improve solubilization time. Mix for 20 min until dissolved.
2. Add 1.0 g/L poloxamer 188 and 2.2 g/L sodium bicarbonate according to Table 1. Mix until dissolved.



**Fig 1.** SFM4CHO medium is available as liquid or powder in pack sizes suitable for small-volume cell culture as well as large-scale bioprocessing applications.

3. Bring vessel to final volume with cell culture-grade water. Allow solution to mix for 20 min.
4. Check pH and osmolality. Expected values:
  - pH 7.0 to 7.4
  - Osmolality 280 to 320 mOsm/kg
5. Sterile filter into desired container using a 0.2 µm sterile filter.

**Table 1.** Sodium bicarbonate supplementation guide

CO <sub>2</sub> environment	Sodium bicarbonate level
Online pH control	0.25 g/L
5% CO <sub>2</sub> incubator	2.20 g/L
10% CO <sub>2</sub> incubator	3.60 g/L

Note: Additional buffering can be achieved by adding 5 to 25 mM HEPES.

## Preparation note

SH30518 does not contain L-glutamine. Recommended concentration: 4 mM.

## General culture recommendations

1. Cultures should be incubated at 37°C in a 5% CO<sub>2</sub> environment.
2. Maintain adapted cells by establishing a mid-logarithmic growth phase subculturing schedule.
3. Suggested seeding density of cultures: 2.0 × 10<sup>5</sup> cells/mL; viability should be > 90%.

## Direct adaptation

1. Transfer cells grown in current medium directly into SFM4CHO at 2.0 × 10<sup>5</sup> cells/mL.
2. When viable cell density reaches 1.0 to 1.5 × 10<sup>6</sup> cells/mL, subculture the cells.
3. Cells should be subcultured every 48 to 96 h.
4. If cell viability drops below 80%, proceed to sequential adaptation.

## Sequential adaptation

Dilute serum-containing medium with an equal volume of SFM4CHO. This preparation will be referred to as the sequential adaptation medium (SAM). Prepare twice the volume of medium needed for the culture vessel in use (i.e., for a T-75 flask using 25 mL of medium, prepare 50 mL of SAM). Prior to each subculture step, warm medium to 37°C.

1. Subculture the cells into SAM at a seeding concentration of 2.0 × 10<sup>5</sup> cells/mL. For best results, the culture should be ~ 70% confluent.
2. When the cells reach a density of 1.0 to 1.5 × 10<sup>6</sup> cells/mL, subculture into an equal mixture of SAM and fresh SFM4CHO at a seeding density of 2.0 × 10<sup>5</sup> cells/mL.

## Cryopreservation

SFM4CHO adapted cells can be cryopreserved in a medium consisting of a 1:1 ratio of fresh and conditioned SFM4CHO medium. To this, add DMSO to a final concentration of 7.5%.

## Quality control testing

Quality control test specifications are listed in Table 2.

**Table 2.** Test specifications

Appearance	Clear yellow solution
Osmolality	280 to 320 mOsm/kg
pH	7.0 to 7.4
Sterility	No growth (bacteria or fungi)
Endotoxin	< 10.0 EU/mL <sup>1</sup>
Application	Growth promotion <sup>1</sup>

<sup>1</sup>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

Table 3 gives an overview of HyClone supplements.

### 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. Each supplement is developed through the Metabolic Pathway Design process and is chemically defined and protein-free with no animal-derived components.

### HyClone LS250 supplement

LS250 is a chemically defined, animal-derived component-free lipid supplement developed to stimulate cell growth and monoclonal antibody (MAb) production in NS0 cell cultures using traditional hybridoma serum-free media.

### HyClone LS1000 supplement

LS1000 supplement is a chemically defined, animal-derived component-free lipid supplement developed to stimulate cell growth and MAb production in NS0 cell cultures using traditional hybridoma serum-free media.

The supplement is formulated using a proprietary complexing process for enhanced cholesterol delivery. LS1000 has been successfully tested in a variety of serum-free medium cultures, including HyClone CDM4NS0 and CDM4MAB media.

**Table 3.** 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
LS250 supplement							●	●	NS0	SH30554
LS1000 supplement								●	NS0	SH30555

\*Animal-derived component-free

## Ordering information

SFM4CHO 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 SFM4CHO liquid medium With L-glutamine	500 mL bottle	SH30549.01
	1000 mL bottle	SH30549.02
	6 × 1000 mL bottles	SH30549.LS
	5 L bag	SH30549.03
	10 L bag	SH30549.04
	20 L bag	SH30549.05
	50 L bag	SH30549.06
	100 L bag	SH30549.07
	200 L bag	SH30549.08
	500 L bag	SH30549.09
HyClone SFM4CHO liquid medium Without L-glutamine	500 mL bottle	SH30548.01
	1000 mL bottle	SH30548.02
	6 × 1000 mL bottles	SH30548.LS
	5 L bag	SH30548.03
	10 L bag	SH30548.04
	20 L bag	SH30548.05
	50 L bag	SH30548.06
	100 L bag	SH30548.07
	200 L bag	SH30548.08
	500 L bag	SH30548.09
HyClone SFM4CHO powder medium Without L-glutamine	1 × 5 L	SH30518.01
	1 × 10 L	SH30518.02
	1 × 50 L	SH30518.03
	1 × 100 L	SH30518.04
	1 × 500 L	SH30518.05
	1 × 1000 L	SH30518.06

Related products	Size	Code number
HyClone Cell Boost kit	6 × 100 g	SH30890
HyClone LS1000 cholesterol supplement	50 mL bottle	SH30554.01
	100 mL bottle	SH30554.02
	500 mL bottle	SH30554.03
	1000 mL bottle	SH30554.04
HyClone LS250 lipid supplement	100 mL bottle	SH30555.01
	500 mL bottle	SH30555.02
	1000 mL bottle	SH30555.03

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