HyClone™ media and supplements

CDM4NS0

HyClone CDM4NS0 is an animal-derived component-free (ADCF), hydrolysate-free cell culture medium. This regulatory-friendly medium is developed through the HyClone Metabolic Pathway Design process (see box) to increase process yields of a variety of NS0 cell clones in the manufacture of monoclonal antibodies (MAbs). CDM4NS0 medium has been formulated to support the needs of NS0 cell clones and as such does not require cholesterol or glutamine synthetase (GS) supplementation. CDM4NS0 medium has been successfully tested in a variety of culture systems, including T-flasks, shaker flasks, and bioreactors using batch and fed-batch strategies. CDM4NS0 is available in liquid and powder formats in user-friendly packaging (Fig 1).

Features of CDM4NS0 medium are

- Hydrolysate-free and animal-derived component-free formulation
- Developed through Metabolic Pathway Design process for high cell yield and MAb production
- Ready-to-use formulation, requires no cholesterol or GS supplementation
- Allows for direct or sequential adaptation
- Designed for large-scale culture applications, including perfusion and fed-batch strategies
- Manufactured from traceable components according to cGMP guidelines

Specifications

- Liquid medium contains poloxamer 188
- Does not contain phenol red
- Does not contain L-glutamine
- Store at 2°C to 8°C away from light
- Protein-free formulation

Fig 1. CDM4NS0 medium is available as liquid or powder in pack sizes suitable for small-volume cell culture as well as large-scale bioprocessing applications.

Metabolic Pathway Design process

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.

Suggested preparation

Reconstitution of CDM4NS0 powder medium

1. While stirring, add 21.5 g/L of CDM4NS0 powder medium to cell culture-grade water (room temperature) at 90% of final preparation volume. If your water source is normally cool, it may 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 0.5 g/L poloxamer 188 and 3.2 g/L sodium bicarbonate. Minimum mixing time: 20 min.
3. Bring vessel to final volume with cell culture-grade water. Allow solution to mix for 10 to 20 min.
4. Check pH and osmolality. Expected values:
   - pH 7.0 to 7.4
   - Osmolality 280 to 310 mOsm/kg
5. Sterile filter into desired container using a 0.2 µm sterile filter.

Preparation notes

CDM4NS0 medium does not contain L-glutamine. Recommended concentration is 6 mM for non-GS NS0 cells. Liquid and powder CDM4HEK293 media should be stored at 2°C to 8°C away from light.

General culture recommendations

1. Cultures should be incubated at 37°C in a 5% CO_2 environment.
2. The caps on culture flasks should be loosened and adequate vessel headspace should be given to provide gas exchange.
3. Seeding densities should be ~ 3.0×10^5 cells/mL after cells are adapted. Cells should typically be subcultured every 3 to 5 days, as necessary.

Direct adaptation

1. Transfer cells grown in current medium directly into CDM4NS0 medium at 5.0×10^5 cells/mL.
2. When viable cell density reaches 2.0 to 4.0×10^6 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

1. Transfer cells grown in current medium into CDM4NS0 medium at a ratio of 1:1 using a seeding density of 5.0×10^5 cells/mL.
2. Incubate culture until two population doublings are observed. Subculture cells by mixing equal volumes of cell suspension in conditioned medium and fresh CDM4NS0 medium (1:1 ratio).
3. Continue to subculture the cells using this method until the previously used medium is reduced below 0.05% concentration and cell viability is > 85%.

Note! A seeding density of 5.0×10^5 cells/mL during adaptation increases success.

Cryopreservation

CDM4NS0 medium adapted cells can be cryopreserved in fresh CDM4NS0 medium supplemented with 10.0% DMSO.

Quality control testing

Quality control test specifications are listed in Table 1.

Table 1. Test specifications[1]

Appearance	Clear yellow solution
Osmolality	280 to 310 mOsm/kg
pH	7.0 to 7.4
Sterility	No growth (bacteria or fungi)
Endotoxin	< 1.0 EU/mL[1]
Application	Growth promotion

[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.

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
LS250 supplement							●	●	NS0	SH30554
LS1000 supplement								●	NS0	SH30555

* Animal-derived component-free

Related products

Table 2 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 (Table 2). 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.

Ordering information

CDM4NS0 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 CDM4NS0 liquid medium Without L-glutamine	500 mL bottle	SH30579.01
	1000 mL bottle	SH30579.02
	5 L bag	SH30579.03
	10 L bag	SH30579.04
	20 L bag	SH30579.05
	50 L bag	SH30579.06
	100 L bag	SH30579.07
	200 L bag	SH30579.08
	500 L bag	SH30579.09
HyClone CDM4NS0 powder medium Without L-glutamine	1 × 5 L HDPE* bottle	SH30578.01
	1 × 10 L HDPE* bottle	SH30578.02
	1 × 50 L HDPE* bottle	SH30578.03
	1 × 100 L HDPE* bottle	SH30578.04
	1 × 500 L polybag/pail	SH30578.05
	1 × 1000 L polybag/pail	SH30578.06

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

* High-density polyethylene

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