



HyClone™ media and supplements

CDM4HEK293

HyClone CDM4HEK293 is a chemically defined cell culture medium optimized for use with human embryonic kidney (HEK) 293 cells. CDM4HEK293 is a protein-free and animal-derived component-free medium that promotes adenovirus and recombinant protein production. This regulatory-friendly medium was developed through the HyClone Metabolic Pathway Design process (see box) to support the extremely high productivity and cell density in suspension cultures. CDM4HEK293 is available in liquid and powder formats in user-friendly packaging (Fig 1).

Features of CDM4HEK293 medium

- Animal-derived component-free
- Chemically defined formulation
- Metabolically designed for high cell yield and recombinant protein/vector production
- Allows for direct or sequential adaptation
- Manufactured from traceable components according to cGMP guidelines

Specifications

- Does not contain phenol red
- Does not contain L-glutamine
- Liquid medium contains poloxamer 188
- Stored at 2°C to 8°C away from light.

Suggested preparation

Reconstitution of CDM4HEK293 powder medium

1. While stirring, add CDM4HEK293 powder medium (SH30859) to cell culture-grade water (20°C to 25°C) at 90% of final preparation volume (19.5 g/L). 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. Medium should be a clear, yellow solution at this point.



Fig 1. CDM4HEK293 medium is available as liquid or powder in pack sizes suitable for small-volume cell culture as well as large-scale bioprocessing applications.

2. Add 1.0 g/L of poloxamer 188 and 2.0 g/L of sodium bicarbonate. Make sure each component has completely dissolved before adding the next component.
3. Bring vessel to final volume with culture-grade water. Allow solution to mix for 10 to 20 min.

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.

- 4. Adjust pH to between 7.0 and 7.4 by adding 1 N NaOH or 1 N HCl drop wise to solution.
- 5. Check osmolality. Expected value is 290 to 340 mOsm/kg.
- 6. Sterile filter into desired container using a 0.2 µm sterile filter.

Preparation notes

CDM4HEK293 powder medium does not contain L-glutamine. Recommended concentration: 4 mM. 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% CO₂ environment.
- 2. Adaptation of HEK 293 cells from adherent and serum-rich conditions is best achieved using a two-step process. First, adaptation to serum-free suspension conditions is achieved using HyClone SFM4HEK293 medium. Once cells have adapted to this serum-free medium they can be directly adapted to CDM4HEK293 medium. Follow the adaptation protocol for SFM4HEK293 medium to adapt cells from serum-containing conditions.

Direct adaptation

- 1. Transfer cell grown in current serum-free medium directly into CDM4HEK293 medium at 3.0×10^5 cells/ mL.
- 2. Passage cells every 3 to 4 days.
- 3. Adaptation is complete once cells have transitioned from a doubling rate of 30 h to a rate of 24 h or less per doubling.

Sequential adaptation

- 1. Transfer cells grown in current medium into CDM4HEK293 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 CDM4HEK293 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%.

Cell maintenance

Maintain adapted cells by establishing a passage schedule that allows the cells to be passed while in log growth phase. HEK 293 cells cultivated in CDM4HEK293 medium should be subcultured every 3 to 4 days (72 to 96 h). The passage schedule and seeding density may be adjusted to optimize performance. The recommended cell seeding density of new cultures for general maintenance is 300 000 cells/mL. The culture viability of an adapted culture should remain greater than 90%. However, during adaptation from serum-containing medium, viabilities can be slightly lower than 90%. Cells should exhibit a population doubling time of approximately 20 to 24 h. If the recommended seed density of 3.0×10^5 cells/mL is used, cultures typically reach approximately 2.5 to 3.5×10^6 cells/mL after 72 h and 5.0 to 6.0×10^6 cells/mL after 96 h. Doubling times during an adaptation period may be higher. Seed stock does not require centrifugation for spent medium removal unless the seeding volume is greater than 50% of the culture working volume. This might occur during adaptation, but should not be the case during general culture maintenance.

Cryopreservation

CDM4HEK293 medium adapted cells can be cryopreserved in a medium consisting of a 1:1 ratio of fresh and conditioned CDM4HEK293 medium supplemented with DMSO at a final concentration of 7.5%.

Quality control testing

Quality control test specifications are listed in Table 1.

Table 1. Test specifications¹

Appearance	Clear yellow solution
Osmolality	290 to 340 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

Supplements

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.

HyCell™ TransFx-H medium

Serum-free, animal-derived component-free medium designed to support the growth of HEK 293 cultures and promote transfection using lipofection or similar methods for transient transfection and production.

Ordering information

CDM4HEK293 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 CDM4HEK293 liquid medium	500 mL bottle	SH30858.01
	1000 mL bottle	SH30858.02
	5 L bag	SH30858.03
	10 L bag	SH30858.04
	20 L bag	SH30858.05
	50 L bag	SH30858.06
	100 L bag	SH30858.07
	200 L bag	SH30858.08
	500 L bag	SH30858.09
	900 L bag	SH30858.10
HyClone CDM4HEK293 powder medium	1 × 5 L HDPE* bottle	SH30859.01
	1 × 10 L HDPE* bottle	SH30859.02
	1 × 50 L HDPE* bottle	SH30859.03
	1 × 100 L HDPE* bottle	SH30859.04
	1 × 500 L polybag/pail	SH30859.05
	1 × 1000 L polybag/pail	SH30859.06

Related products	Size	Code number
HyClone HyCell TransFx-H liquid medium Without L-glutamine Without poloxamer 188	500 mL PETE [†] bottle	SH30939.01 [‡]
	1 L PETE [†] bottle	SH30939.02 [§]
	1 L bag	SH30939.03 [‡]
	5 L bag	SH30939.04 [‡]
	10 L bag	SH30939.05 [‡]
	20 L bag	SH30939.06 [‡]
	50 L bag	SH30939.07 [‡]
	100 L bag	SH30939.08 [‡]
	200 L bag	SH30939.09 [‡]
	500 L bag	SH30939.10 [‡]
HyClone HyCell TransFx-H powder medium Without L-glutamine Without poloxamer 188	5 L in HDPE* bottle	SH30944.01 [§]
	10 L in HDPE* bottle	SH30944.02 [§]
	50 L in HDPE* bottle	SH30944.03 [§]
	100 L in HDPE* bottle	SH30944.04 [§]
	500 L in polybag/pail	SH30944.05 [§]
	1000 L in polybag/pail	SH30944.06 [§]
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

[†] Polyethylene terephthalate

[‡] Item is made to order. Lead times and minimum order quantities apply.

[§] Item in stock

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