

## HyClone<sup>™</sup> media and supplements

# SFM4Transfx-293

HyClone SFMTransfx-293 medium is a serum-free and animal-derived component-free medium designed to support the growth of HEK 293 cell cultures and promote transfection using lipofection, polymer, or similar methods. This regulatory-friendly medium was developed through the HyClone Metabolic Pathway Design process (see box) to support high transfection efficiency, cell density, and productivity in suspension cultures. SFM4Transfx-293 medium is available in liquid and powder formats in userfriendly packaging (Fig 1).

#### Key features of SFMTransfx-293 medium include

- Animal-derived component-free
- Supports high transfection efficiency
- Designed for high cell yield and recombinant protein/ vector production
- Allows for direct or sequential adaptation
- Manufactured from traceable components according to cGMP (21 CFR 820) guidelines

# **Specifications**

- Does not contain L-glutamine
- Does not contain phenol red
- Liquid medium contains poloxamer 188

## **Product handling**

Store SFM4Transfx-293 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.



**Fig 1.** SFM4Transfx-293 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 on a variety of factors, such as cell line, specific clones, media, and feeds, as well as processes to maximize viable cell densities and productivity. Our experts in medium design and development know and understand these factors at the metabolic level. They evaluate each metabolic profile, understanding nutritional demands and waste creation, to make sure the correct nutrient type and quantity is used to minimize waste and resultant cell toxicity. Our experts use their understanding of metabolic pathways to optimize media for enhanced viable cell densities and productivity. Once the medium has been optimized using this Metabolic Pathway Design process, our scientists can help you devise an effective cell culture strategy using a combination of media and feeds to further enrich productivity and reduce process inefficiencies.

# Suggested preparation Reconstitution of SFM4Transfx-293 powder medium

- While stirring, add SFM4Transfx-293 powder medium to cell culture-grade water (20°C to 25°C) at 90% of final preparation volume (19.5 g/L). Mix until dissolved. Medium should be a clear, golden solution at this point. If your water source is normally cool, it might be useful to adjust the water temperature. Using warmer room temperature water (22°C to 25°C) will improve dissolution time. Mix for 20 min or until dissolved.
- 2. Add 1.0 g/L poloxamer 188 and 2.0 g/L sodium bicarbonate. Ensure each component has completely dissolved before adding the next component.
- 3. Bring vessel to final volume with cell culture-grade water. Allow solution to mix for 10 to 20 min.
- 4. Adjust pH to between 7.0 and 7.2 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  $\mu m$  sterile filter.

## **Preparation notes**

SFM4Transfx-293 powder medium does not contain L-glutamine. Recommended concentration: 4 mM.

# **General culture recommendations**

- 1. Cultures should be incubated at 37°C in a 5%  $CO_2$  environment.
- 2. Adaptation of HEK 293 cells from adherent and serumrich conditions is best achieved using a two-step process. First, adaptation to serum-free suspension conditions is achieved using HyClone SFM4HEK293 medium or HyClone CDM4HEK293 medium. Once cells have adapted to this serum-free medium, they can be directly adapted to SFM4Transfx-293 medium.

## **Direct adaptation**

- 1. Transfer cell grown in current serum-free medium directly into SFM4Transfx-293 medium at  $3.0 \times 10^5$  cells/mL.
- 2. Passage cells every 3 to 4 days.
- 3. Adaptation is complete once cells have transitioned to a rate of 24 h per doubling.

## 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 SFM4Transfx-293 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  $3 \times 10^5$  cells/mL. The culture viability of an adapted culture should remain greater than 90%. During adaptation from serum-containing medium, however, viabilities might be slightly lower than 90%. Cells should exhibit a population doubling time of approximately 24 h. If the recommended seed density of  $3.0 \times 10^5$  cells/mL is used, cultures typically reach approximately 2.5 to  $3.5 \times 10^6$  cells/mL after 72 h and 5.0 to  $6.0 \times 10^6$  cells/mL after 96 h. Doubling times during an adaptation period might 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, which can occur during adaptation but should not be the case during general culture maintenance.

## Cryopreservation

SFM4Transfx-293 medium adapted cells can be cryopreserved in a medium consisting of a 1:1 ratio of fresh and conditioned SFM4Transfx-293 medium. To this, add DMSO to 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
рН	7.0 to 7.4
Sterility	No growth (bacteria or fungi)
Endotoxin	≤ 5.0 EU/mL <sup>1</sup>
Application	Growth promotion

<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 media 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	Product code
Cell Boost 1 Supplement (R05.2)	•	•	٠						HEK293 CHO	SH30584
Cell Boost 2 Supplement (R15.4)	•		•						PER.C6™ CHO	SH30596
Cell Boost 3 Supplement (JM3.5)	•	•	•	•		٠			Hybridoma Myeloma	SH30825
Cell Boost 4 Supplement (PS307)	•	•	•	•	•		•	•	СНО	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<sup>™</sup> 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 componentfree 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.

#### HyClone CDM4HEK293 medium

CDM4HEK293 medium is chemically defined and contains no animal-derived components. The medium supports the growth of HEK 293 cultures and promotes adenovirus and recombinant protein production.

# **Ordering information**

Product	Size	Product code
HyClone SFM4Transfx-293	500 mL bottle	SH30860.01
liquid medium	1000 mL bottle	SH30860.02
	6 × 1000 mL bottle	SH30860.LS
	5 L bag	SH30860.04
	10 L bag	SH30860.05
	20 L bag	SH30860.06
	50 L bag	SH30860.07
	100 L bag	SH30860.08
	200 L bag	SH30860.09
	500 L bag	SH30860.10
	900 L bag	SH30860.11
HyClone SFM4Transfx-293	1 × 5 L*	SH30861.01
powder medium	1 × 10 L*	SH30861.02
	1 × 50 L*	SH30861.03
	$1 \times 100 L^{\dagger}$	SH30861.04
	$1 \times 500 L^{\dagger}$	SH30861.05
	$1 \times 1000 \ L^{\dagger}$	SH30861.06

Related products	Size	Product code
HyClone Cell Boost Kit	6 × 100 g	SH30890
HyClone LS1000	50 mL bottle	SH30554.01
cholesterol supplement	100 mL bottle	SH30554.02
	500 mL bottle	SH30554.03
	1000 mL bottle	SH30554.04
HyClone LS250	100 mL bottle	SH30555.01
lipid supplement	500 mL bottle	SH30555.02
	1000 mL bottle	SH30555.03
HyClone CDM4HEK293		
liquid medium		SH30858
HyClone CDM4HEK293		
powder medium		SH30859

\* High-density polyethylene (HDPE) bottle

† Polybag/pail

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