PF-CHO LS and PF-CHO MPS

HYCLONE MEDIA AND SUPPLEMENTS

HyClone[™] PF-CHO LS liquid and PF-CHO MPS powder media are versatile and protein-free. The media are developed through the HyClone Metabolic Pathway Design process (see box) to support the growth of multiple Chinese hamster ovary (CHO) cell clones and production of a variety of recombinant proteins with minimal adaptation.

PF-CHO LS and PF-CHO MPS media are well-suited for cost-effective manufacturing of recombinant proteins for academic and industrial applications such as genomics, proteomics, and *in vitro* diagnostics, as well as for screening and validation of target molecules and manufacturing of biologics.

PF-CHO LS and PF-CHO MPS media are designed to support the dihydrofolate reductase (DHFR) selection/amplification system. The media have been successfully tested in a variety of cell culture systems, including T-flasks, spinner flasks, and bioreactors. PF-CHO LS liquid and PF-CHO MPS are available in liquid and powder formats in user-friendly packaging (Fig 1).

Key features of PF-CHO LS liquid and PF-CHO MPS powder media:

- Designed for high cell yield and recombinant protein production
- Allow for direct or sequential adaptation
- Support growth in multiple cell culture systems
- Manufactured from traceable components according to cGMP (21 CFR 820) guidelines

Specifications

- Protein-free
- Do not contain phenol red
- PF-CHO LS liquid medium contains 4 mM L-glutamine and 0.1% poloxamer 188
- PF-CHO MPS powder medium does not contain L-glutamine or poloxamer 188



Fig 1. PF-CHO LS and PF-CHO MPS are protein-free media available in packaging and sizes for applications ranging from small-volume cell culture to 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.



Product handling

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

Suggested preparation

Reconstitution of PF-CHO MPS powder medium

PF-CHO MPS powder medium is a two-part powder comprising a base powder (SH30335) and a main powder (SH30334).

- While stirring, add PF-CHO MPS base powder to cell culturegrade water at 80% of final preparation volume (10.4 g/L). Mix until dissolved. 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. Adjust pH to 7.0 using 5 N HCL. Mix for at least 10 min.
- 3. After the base powder has dissolved, add the PF-CHO MPS main powder to vessel (6.0 g/L). Mix until dissolved.
- 4. Add sodium bicarbonate according to Table 1 and mix until dissolved.
- 5. Add 1.0 g/L poloxamer 188. Mix until dissolved.
- 6. Bring vessel to final volume with cell culture-grade water. Allow solution to mix for 20 min.
- 7. Check pH and osmolality. Expected values:
 - pH 7.2 to 7.8
 - Osmolality 300 to 340 mOsm/kg
- 8. Sterile filter into desired container using a 0.2 µm sterile filter.

Preparation notes

PF-CHO MPS powder medium does not contain L-glutamine. Recommended concentration: 4 mM.

Table 1. Sodium bicarbonate supplementation guide

| CO ₂ environment | Sodium bicarbonate level |
|-------------------------------|--------------------------|
| Online pH control | 0.25 g/L |
| 5% CO ₂ incubator | 2.20 g/L |
| 10% CO ₂ incubator | 3.60 g/L |

General culture recommendations

- 1. Cultures should be incubated at 37°C in a 5% CO_2 environment.
- 2. Maintain adapted cells by establishing mid-logarithmic growth phase subculture schedule.
- Suggested seeding density of cultures: 2.0 × 10⁵ cells/mL, viability should be > 90%.

Direct adaptation

- 1. Transfer cells grown in current medium directly into PF-CHO LS medium at 2.0×10^5 cells/mL.
- 2. When viable cell density reaches 1.0 to 1.5×10^6 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

Dilute serum-containing medium with an equal volume of PF-CHO LS medium. 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, warm medium to 37°C.

- 1. Subculture the cells into SAM at a seeding concentration of 2.0×10^5 cells/mL. For best results, the culture should be ~ 70% confluent.
- 2. When the cells reach a density of $1.0 \text{ to } 1.5 \times 10^6 \text{ cells/mL}$, subculture into an equal mixture of SAM and fresh PF-CHO LS medium at a seeding density of $2.0 \times 10^5 \text{ cells/mL}$.

Cryopreservation

PF-CHO LS medium adapted cells can be cryopreserved in a medium consisting of a 1:1 ratio of fresh and conditioned PF-CHO LS medium. To this, add DMSO at a final concentration of 7.5%.

Quality control testing

Quality control test specifications are listed in Table 2.

Table 2. Test specifications¹

| Appearance | Clear solution |
|-------------|-------------------------------|
| Osmolality | 300 to 340 mOsm/kg |
| рН | 7.2 to 7.8 |
| Sterility | No growth (bacteria or fungi) |
| Endotoxin | < 10.0 EU/mL ¹ |
| Application | Growth promotion |

¹ Refer to certificate of analysis for actual results.

Table 3. 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) | • | • | • | | | | | | 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) | • | • | • | • | • | | • | • | 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 | | | • | - | | | ٠ | • | NS0 | SH30554 |
| LS1000 supplement | •••••• | | •••••• | | • | | •••••• | • | NS0 | SH30555 |

* Animal-derived component-free

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.

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 proteinfree 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.

Ordering information

HyClone PF-CHO LS medium is manufactured in homogenous liquid lot sizes up to 10 000 L and PF-CHO MPS powder in lot sizes up to 250 000 L.

| Product | Size | Product code |
|--|-------------------------|--------------|
| HyClone PF-CHO LS | 1000 mL bottle | SH30359.02 |
| liquid medium With L-glutamine and poloxamer 188 | 20 L bag | SH30359.03 |
| | 50 L bag | SH30359.04 |
| | 100 L bag | SH30359.05 |
| | 200 L bag | SH30359.06 |
| | 500 L bag | SH30359.07 |
| HyClone PF-CHO MPS powder medium Without L-glutamine or poloxamer 188 | 1 × 5 L* | SH30333.01 |
| | 1 × 10 L | SH30333.02 |
| | 1 × 50 L* | SH30333.03 |
| | 1 × 100 L* | SH30333.04 |
| | 1 × 500 L [†] | SH30333.05 |
| | 1 × 1000 L [†] | SH30333.06 |
| | 1 × 5000 L [†] | SH30333.06 |

* High-density polyethylene (HDPE) bottle

† Polybag/pail

| Related products | Product code | | |
|---------------------------------------|--------------|--|--|
| HyClone CDM4CHO medium | SH30557 | | |
| HyCell CHO Liquid Medium with HT | SH30934 | | |
| HyCell CHO Powder Medium with HT | SH30948 | | |
| HyCell CHO Liquid Medium without HT | SH30949 | | |
| HyCell CHO Powder Medium without HT | SH30933 | | |
| HyClone Cell Boost kit | SH30890 | | |
| HyClone LS1000 cholesterol supplement | SH30554 | | |
| HyClone LS250 lipid supplement | SH30555 | | |

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