# ADCF-MAb

## HYCLONE MEDIA AND SUPPLEMENTS

HyClone™ ADCF-MAb cell culture medium is protein-free and contains no animal-derived components. This regulatory-friendly medium is developed through the HyClone Metabolic Pathway Design process (see box) to increase the process yields for the manufacture of antibodies and antibody fragments for therapeutic use in a variety of engineered hybridoma and myeloma cell lines.

ADCF-MAb liquid medium contains poloxamer 188 and L-glutamine, and does not contain phenol red. Liquid medium is also available without L-glutamine to support the glutamine synthetase (GS) gene expression system. ADCF-MAb powder medium does not contain poloxamer 188, L-glutamine, phenol red, or sodium bicarbonate. ADCF-MAb is available in liquid and powder formats in user-friendly packaging (Fig 1).

Key features of ADCF-MAb medium include

- Animal-derived component-free (ADCF) formulation
- Designed for high cell yield and monoclonal antibody (MAb) production
- Allows for direct or sequential adaptation
- Component traceability
- Manufactured according to cGMP guidelines

## Specifications

- Protein-free
- Does not contain phenol red
- Liquid medium contains poloxamer 188 and L-glutamine
- Powder medium does not contain poloxamer 188 or L-glutamine
- Developed through the Metabolic Pathway Design process

### *Product handling*

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




**Fig 1.** ADCF-I 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.

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# Suggested preparation

### *Reconstitution of ADCF-MAb powder medium*

1. While stirring, add 19.9 g/L of ADCF-MAb powder medium to cell culture-grade water 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 or until dissolved.
2. Add 1.0 g/L poloxamer 188 and 1.6 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 340 mOsm/kg
5. Sterile filter into desired container using a 0.2 µm sterile filter.

### *Preparation notes*

ADCF-MAb powder medium does not contain L-glutamine. Recommended concentration: 4 to 6 mM.

# General culture recommendations

1. Cultures should be incubated at 37°C and 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 ~ $1.0 \times 10^5$ cells/mL. Cells should typically be subcultured every 2 to 4 days, as necessary.

### *Direct adaptation*

1. Transfer cells grown in current medium directly into ADCF-MAb medium at $2.0 \times 10^5$ cells/mL. Note: increasing the seeding density at the onset of adaptation can improve success ratios.
2. When viable cell density reaches 1.0 to $1.5 \times 10^6$ 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 ADCF-MAb medium at a ratio of 1:1 using a seeding density of $2.0 \times 10^5$ 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 ADCF-MAb 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%.

### *Cryopreservation*

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

# Quality control testing

Quality control test specifications are listed in Table 1.

**Table 1.** Test specifications[1]

| | |
|---|---|
| Appearance | Clear solution |
| Osmolality | 286 to 336 mOsm/kg |
| pH | 7.0 to 7.4 |
| Sterility | No growth (bacteria or fungi) |
| Endotoxin | < 10.0 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.

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**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 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.

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# Ordering information

ADCF-MAb 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 ADCF-MAb liquid medium<br>With L-glutamine | 500 mL bottle | SH30349.01 |
| | 1000 mL bottle | SH30349.02 |
| | 5 L bag | SH30349.03 |
| | 10 L bag | SH30349.04 |
| | 20 L bag | SH30349.05 |
| | 50 L bag | SH30349.06 |
| | 100 L bag | SH30349.07 |
| | 200 L bag | SH30349.08 |
| HyClone ADCF-MAb liquid medium<br>Without L-glutamine | 500 mL bottle | SH30547.01 |
| | 1000 mL bottle | SH30547.02 |
| | 5 L bag | SH30547.03 |
| | 10 L bag | SH30547.04 |
| | 20 L bag | SH30547.05 |
| | 50 L bag | SH30547.06 |
| | 100 L bag | SH30547.07 |
| | 200 L bag | SH30547.08 |
| HyClone ADCF-MAb powder medium<br>Without L-glutamine | 1 × 5 L* | SH30635.01 |
| | 1 × 10 L* | SH30635.02 |
| | 1 × 50 L* | SH30635.03 |
| | 1 × 100 L* | SH30635.04 |
| | 1 × 500 L† | SH30635.05 |
| | 1 × 1000 L† | SH30635.06 |

* High-density polyethylene (HDPE) bottle
† Polybag/pail

| 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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