

## IS MAB-CD

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Catalog #	Product	Size
91104	IS MAB-CD	1 L liquid

\*Additional packaging sizes are available upon request for liquid and powder formats.

### Intended Use

This product is for research or further manufacturing use only. Not for injection or diagnostic procedures.

### Product Description

IS MAB-CD medium is a chemically-defined medium formulated specifically for the growth of hybridoma and myeloma cell lines for recombinant monoclonal antibody production.

### Formula

IS MAB-CD medium is provided without L-Glutamine to extend shelf life and to allow the use of L-Glutamine feeding strategies. The recommended L-Glutamine concentration to add is 8 mM (40 mL per liter of a 200 mM L-Glutamine solution, Catalog #9317). The medium should be used without L-Glutamine if the GS selection system is being used. IS MAB-CD medium contains no protein hydrolysates. This medium contains no antibiotics or antimycotics.

### Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis, which is available at [www.irvinesci.com](http://www.irvinesci.com) or upon request.

### Storage Instructions and Stability

2° to 8°C, protected from light. Do not use after the indicated expiration date.

#### Indications of Deterioration

Do not use if solution is cloudy or contains precipitates.

### Precautions

Handle using aseptic techniques. Cells grown in serum-free conditions are more sensitive to changes in pH, toxic substances, dissociation agents and the use of selective drugs. Frequent monitoring is suggested.

## Directions for Use

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### ADAPTATION

#### I. Direct Adaptation from Serum-Free Hybridoma Media to IS MAB-CD

In many cases, hybridoma cells may be subcultured from a serum-free medium (e.g., IS MAB-V) directly into IS MAB-CD.

1. Dispense IS MAB-CD medium into a culture vessel and equilibrate to 37°C and 5% CO<sub>2</sub>.
2. Passage hybridoma cells from serum-free culture into IS MAB-CD at 3x10<sup>5</sup> viable cells/mL. It is important that cells be in the logarithmic phase of growth with at least 90% viability before passaging.
3. Incubate cultures at 37°C and 5% CO<sub>2</sub> until the viable cell density reaches 1x10<sup>6</sup> cells/mL.
4. Subculture into fresh IS MAB-CD medium at 2x10<sup>5</sup> cell/mL starting density.
5. Maintain cells in IS MAB-CD for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

#### II. Sequential adaptation from serum-free media to IS MAB-CD

Sequential adaptation may be used if direct adaptation is troublesome.

1. Dispense the original serum-free medium and IS MAB-CD medium in a 1:1 ratio into an appropriate culture vessel and equilibrate to 37°C and 5% CO<sub>2</sub>.
2. Passage hybridoma cells from serum-free culture into the blended medium (step 1) at 3x10<sup>5</sup> viable cells/mL. It is important that cells be in the logarithmic phase of growth with at least 90% viability before passaging.
3. Incubate cultures at 37°C and 5% CO<sub>2</sub> until the viable cell density reaches 1x10<sup>6</sup> cells/mL.
4. Subculture at 3x10<sup>5</sup> cells/mL starting density into fresh medium prepared in a 1:3 ratio of original serum-free medium to IS MAB-CD medium.
5. Repeat steps 3 and 4 with sequential dilution ratios of 1:7, 1:15, and 0:1 of the original serum-free medium and IS MAB-CD. If the cells look unhealthy or the growth rate declines significantly at a particular step of adaptation, maintain the cells for an additional passage in the media ratio of the previous step before subculturing into the next ratio.
6. Maintain cells in IS MAB-CD for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

#### III. Sequential adaptation from serum-supplemented media to IS MAB-CD

1. The direct transfer of cells from serum-supplemented media to IS MAB-CD medium is not recommended. Sequential adaptation can be achieved by gradual weaning of cell cultures from a serum-supplemented medium to IS MAB-CD medium.

2. Cells can be adapted to IS MAB-CD medium by gradually reducing the serum concentration using the sequential ratios of 1:1, 1:3, 1:7, 1:15 and 0:1 of serum-supplemented medium and IS MAB-CD medium. Cells should be grown and subcultured at the densities previously described in Section II (Sequential Adaptation from Serum-Free Media to IS MAB-CD) above.

## **CRYOPRESERVATION**

Viable cell banks may conveniently be created by freezing cells in 90% IS MAB-CD + 10% DMSO. No other additions are necessary.

### Freezing

1. Use cultures that are in logarithmic growth with high viabilities (> 85%).
2. Centrifuge cells for 5 minutes at 200 g.
3. Resuspend in cold (2-8°C) 90% IS MAB-CD, 10% DMSO to a density of  $1 \times 10^7$  viable cells/mL.
4. Aliquot into sterile cryovials.
5. Gradually lower the temperature of the vials to below  $-80^\circ\text{C}$  at a rate of  $-1^\circ\text{C}/\text{minute}$ .
6. Store vials in liquid nitrogen freezer.

### Thawing

1. Thaw frozen vial rapidly in a  $37^\circ\text{C}$  water bath.
2. Transfer the cell suspension to a culture flask with fresh IS MAB-CD medium to achieve an initial cell density of  $3 \times 10^5$  viable cells/mL.
3. Incubate cultures at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$  until the viable cell density reaches  $1 \times 10^6$  cells/mL.
4. Subculture into fresh IS MAB-CD medium at  $2 \times 10^5$  cells/mL starting density.

## Technical Support

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### CONTACT US

For more information or assistance contact Customer Service at:

- Email: [fisitmrequest@fujifilm.com](mailto:fisitmrequest@fujifilm.com)
- Direct line: +1 800 577 6097

### WEBSITE RESOURCES

Visit the website at [www.irvinesci.com](http://www.irvinesci.com) for technical resources and information including:

- Safety Data Sheets (SDS)
- Certificate of Analysis (CoA) (when available)
- FAQs
- Product literature
- Complete list of offices and contact information by country

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