IS MAB-CD™

Catalog No. 91104	1 L Liquid
Catalog No. 94105	10 L Powder

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.

INTENDED USE

For further manufacturing use. IS MAB-CD is a growth medium for the production of recombinant monoclonal antibodies.

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.

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.

Storage
2º to 8º 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.

QUALITY ASSURANCE

IS MAB-CD medium is tested as specified on a lot-specific Certificate of Analysis, which is available upon request.

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 37ºC and 5% CO_2.
2. Passage hybridoma cells from serum-free culture into IS MAB-CD at $3x10^5$ 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 37º C and 5% CO_2 until the viable cell density reaches $1x10^6$ cells/mL.
4. Subculture into fresh IS MAB-CD medium at $2x10^5$ 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 37º and 5% CO_2.
2. Passage hybridoma cells from serum-free culture into the blended medium (step 1) at $3x10^5$ 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 37ºC and 5% CO_2 until the viable cell density reaches $1x10^6$ cells/mL.
4. Subculture at $3x10^5$ 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.

PREPARATION FROM POWDER

1. Measure appropriate volume of WFI Quality Water (I.S. Catalog No. 9309) into a mixing vessel.
2. Add 17.92 g/L of 94105 powdered medium.
3. Add 1.2 g/L of L-Glutamine if required.
4. Mix thoroughly until powdered medium is completely dissolved.
5. Add 2.2 g/L of Sodium Bicarbonate.
6. Mix until Sodium Bicarbonate is completely dissolved. **CAUTION**: Do not overmix. Overmixing may cause Carbon Dioxide degassing.
7. Filter into a sterile vessel.

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 (2-8º C) 90% IS MAB-CD, 10% DMSO to a density of $1x10^7$ viable cells/mL.
4. Aliquot into sterile cryovials.
5. Gradually lower the temperature of the vials to below –80º C at a rate of –1º C/minute.
6. Store vials in liquid nitrogen freezer.

Thawing

1. Thaw frozen vial rapidly in a 37º 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 $3x10^5$ viable cells/mL.
3. Incubate cultures at 37º C and 5% CO_2 until the viable cell density reaches $1x10^6$ cells/mL.
4. Subculture into fresh IS MAB-CD medium at $2x10^5$ cells/mL starting density.

Also Available from IRVINE SCIENTIFIC

IS MAB-V™ **Catalog No. 9199**
IS MAB-V is a serum-free medium optimized for the production of recombinant monoclonal antibodies. This medium is free of any components derived from human, bovine, or other mammalian sources.

IS 293-V™ **Catalog No. 91107**
Irvine Scientific has developed IS 293-V, a new animal-component-free medium formulated specifically for the growth of HEK-293 cells. This formulation supports long term and high density cell growth along with high levels of adenovirus or recombinant protein production.

IS CHO-V™ **Catalog No. 9197**
IS CHO-V-GS™ **Catalog No. 9198**
IS CHO-CD™ **Catalog No. 91119**
IS CHO-CD4™ **Catalog No. 91100**
IS CHO-V and IS CHO-V-GS media are serum-free media optimized for the production of recombinant proteins in Chinese Hamster Ovary (CHO) cells. These media are free of any components derived from human, bovine, or other mammalian sources. IS CHO-CD and IS CHO-CD4 media are chemically defined.

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