

IS CHO-CD4™ MEDIUM

Catalog No. 91100 500 mL, 1 L & 20 L Liquid
Catalog No. 94104 10 L Powder

INTENDED USE

IS CHO-CD4™ Medium is a chemically-defined medium optimized for the production of recombinant proteins in Chinese Hamster Ovary (CHO) cells.

QUALITY ASSURANCE

All Quality Control test results are reported on a lot specific Certificate of Analysis which is available upon request.

FORMULA

IS CHO-CD4 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 (40mL 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 CHO-CD4 medium has been designed without Hypoxanthine and Thymidine for use with the dihydrofolate reductase (dhfr) and other selection systems. IS CHO-CD4 medium contains no protein hydrolysates or any other undefined components. This medium contains no antibiotics or antimycotics.

STORAGE INSTRUCTIONS AND STABILITY

Handle using aseptic techniques to avoid contamination.

Store at 2–8° C, away from light.

Do not use after the assigned expiration date.

Indications of deterioration: Do not use if cloudy or if solution precipitates.

DIRECTIONS FOR USE

ADAPTATION

I. Direct Adaptation from Serum-Supplemented Media to IS CHO-CD4

In most cases, CHO cells may be subcultured from a serum-supplemented medium (e.g., Ham's F-12/DME + 10% FBS) directly into IS CHO-CD4.

1. Dispense IS CHO-CD4 medium into a culture vessel and equilibrate to 37° C and 5% CO₂.
2. Passage CHO cells from serum-supplemented culture into IS CHO-CD4 at 3x10⁵ 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% CO₂ until the viable cell density reaches 1x10⁶ cells/mL.
4. Subculture into fresh IS CHO-CD4 medium at 2x10⁵ cell/mL starting density.
5. Maintain cells in IS CHO-CD4 for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

II. Sequential Adaptation from Serum-Supplemented Media to IS CHO-CD4

Sequential adaptation may be used if direct adaptation is troublesome.

1. Dispense the original serum-supplemented medium and IS CHO-CD4 medium in a 3:1 ratio into an appropriate culture vessel and equilibrate to 37° and 5% CO₂.
2. Passage CHO cells from serum-supplemented culture into 3:1 IS CHO-CD4 at 3x10⁵ 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% CO₂ until the viable cell density reaches 1x10⁶ cells/mL.
4. Subculture at 3x10⁵ cells/mL starting density into fresh medium prepared in a 2:1 ratio of original serum-supplemented medium to IS CHO-CD4 medium.
5. Repeat steps 3 and 4 with sequential dilution ratios of 1:1, 1:2, 1:4, and 0:1 of the original serum-supplemented medium and IS CHO-CD4. 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 CHO-CD4 for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

PREPARATION FROM POWDER (Catalog No. 94104)

1. Measure appropriate volume of WFI Quality Water (I.S. Catalog No. 9309) into a mixing vessel.
2. Add 19.00 g/L of 94104 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 CHO-CD4 + 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 CHO-CD4, 10% DMSO to a density of 1x10⁷ viable cells/mL.
4. Aliquot into sterile cryovials.
5. Gradually lower the temperature of the vials to below –80° C at a rate of –1° C/minute.
6. Store vials in liquid nitrogen freezer.

Thawing

1. Thaw frozen vial rapidly in a 37° C water bath.
2. Transfer the cell suspension to a culture flask with fresh IS CHO-CD4 medium to achieve an initial cell density of 3x10⁵ viable cells/mL.
3. Incubate cultures at 37° C and 5% CO₂ until the viable cell density reaches 1x10⁶ cells/mL.
4. Subculture into fresh IS CHO-CD4 medium at 2x10⁵ cells/mL starting density.

PRECAUTIONS AND WARNINGS

Do not use any bottle of medium which shows evidence of particulate matter or cloudiness.

IS CHO-CD4 is a trademark of Irvine Scientific.

ALSO AVAILABLE FROM IRVINE SCIENTIFIC

IS CHO-V™ Catalog No. 9197
IS CHO-V-GS™ Catalog No. 9198
IS CHO-CD™ Catalog No. 91109

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 medium is a chemically defined medium.

IS 293-V™ Catalog No. 91107
Irvine Scientific has developed IS 293-V, an 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 MAB-V™ Catalog No. 9199
Irvine Scientific has developed IS MAB-V, an animal-component-free medium to support long term, high density cell growth along with high levels of antibody production.



IrvineScientific®

2511 Daimler Street, Santa Ana, California 92705-5588
Telephone: 1 949 261 7800 • 1 800 437 5706
Fax: 1 949 261 6522 • www.irvinesci.com

PN40766 Rev. 0