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## QUALITY ASSURANCE

IS CHO-CD™ is a trademark of Irvine Scientific. All testing results are reported on a lot specific Certificate of Analysis, which is available upon request.

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Also Available for CHO Cells:

**IS CHO-V™** **Catalog No. 9197**

A serum-free medium with only non-mammalian origin components. Formulated for dihydrofolate reductase (dhfr) selection.

**IS CHO-V-GS™** **Catalog No. 9198**

Similar to IS CHO-V™, but designed for use with the GS selection system.

**IS CHO™** **Catalog No. 91109**

Serum-free medium containing BSA and transferrin.



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## IS CHO-CD™ MEDIUM

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**Catalog #91119**

**1 L Liquid**

**Catalog #94111**

**10 L Powder**

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### DESCRIPTION

IS CHO-CD™ is a chemically-defined medium optimized for the production of recombinant protein in Chinese Hamster Ovary (CHO) cells. The formula contains only defined components of non-animal origin. IS CHO-CD medium has been designed without Hypoxanthine, Thymidine or L-glutamine for use with the dihydrofolate reductase (dhfr), Glutamine Synthetase (GS) or other selection systems.

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### INTENDED USE

For further manufacturing use. IS CHO-CD is a growth medium for the production of recombinant proteins in CHO cells.

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### FORMULA

IS CHO-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. The medium should be used without L-Glutamine if the GS selection system is being used. IS CHO-CD medium has been designed without Hypoxanthine and Thymidine for use with the dihydrofolate reductase (dhfr) and other selection systems. This medium contains no antibiotics or antimycotics. This medium contains 10 mg/L human recombinant insulin.

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### PRECAUTIONS

Handle using aseptic techniques to avoid contamination.

Storage: Store at 2-8° C, protected from light. Do not use after the assigned expiration date.

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

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## INSTRUCTIONS FOR USE

### Adaptation

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

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

1. Dispense IS CHO-CD medium into an appropriate culture vessel and equilibrate to 37° C and 5% CO<sub>2</sub>.
2. Passage CHO cells from serum-free culture into IS CHO-CD at 3 X 10<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 1 X 10<sup>6</sup> cells/mL.
4. Subculture into fresh IS CHO-CD medium at 2 X 10<sup>5</sup> cell/mL starting density.
5. Maintain cells in IS CHO-CD for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

#### II. Sequential Adaptation from Serum-Free Media to IS CHO-CD

Sequential adaptation may be used if direct adaptation is troublesome.

1. Dispense the original serum-free medium and IS CHO-CD medium in a 3:1 ratio into an appropriate culture vessel and equilibrate to 37° C and 5% CO<sub>2</sub>.
2. Passage CHO cells from serum-free culture into 3:1 IS CHO-CD at 3 X 10<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 1 X 10<sup>6</sup> cells/mL.
4. Subculture at 3 X 10<sup>5</sup> cells/mL starting density into fresh medium prepared in a 2:1 ratio of original serum-free medium to IS CHO-CD 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-free medium and IS CHO-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 CHO-CD for several passages, subculturing twice weekly to allow complete adaptation and assure optimum performance.

#### III. Sequential Adaptation from Serum-Supplemented Media to IS CHO-CD

1. The direct transfer of cells from serum-supplemented media to IS CHO-CD medium is not recommended. Sequential adaptation can be achieved by gradual weaning of cell cultures from a serum-supplemented medium to IS CHO-CD medium.
2. Cells can be adapted to IS CHO-CD medium by gradually reducing the serum concentration using the sequential ratios of 3:1, 2:1, 1:1, 1:2, 1:4, 0:1 of serum-supplemented medium and IS CHO-CD medium. Cells should be grown and subcultured at the densities previously described in Section II above.

#### Reconstitution of Powder Media

The complete powder IS CHO-CD should be reconstituted at 17.50 g/L with WFI or cell culture grade water to slightly less than the final volume. Add 1.2 g/L L-Glutamine to the medium and mix until completely dissolved. Sodium bicarbonate should be added to a final concentration of 2.2 g/L. Adjust the pH of the medium following reconstitution and addition of sodium bicarbonate to 7.2 +/- 0.1 with 1 N HCl or 1 N NaOH. After all adjustments have been made, bring the medium to its final volume. Sterile filter the reconstituted medium into sterile, re-closeable containers before use.

#### Cell Banking

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

#### Freezing

1. Centrifuge cells for 5 minutes at 200 g.
2. Resuspend in cold (2-8 °C) 90% IS CHO-CD, 10% DMSO to a density of 1 X 10<sup>7</sup> viable cells/mL...
3. Aliquot into sterile cryovials.
4. Gradually lower the temperature of the vials to below -80° C at a rate of -1° C/minute.
5. 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-CD medium to achieve an initial cell density of 3 X 10<sup>5</sup> viable cells/mL.
  3. Incubate cultures at 37° C and 5% CO<sub>2</sub> until the viable cell density reaches 1 X 10<sup>6</sup> cells/mL.
  4. Subculture into fresh IS CHO-CD medium at 2 X 10<sup>5</sup> cells/mL starting density.
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