CHO-S Cells  
Cat. No. 11619-012

Shipping and Storage  
Cells are supplied in a cryogenic vial containing 1.5 x 10⁷ viable cells in a volume of 1.5 mL. **Store in liquid nitrogen (vapor phase).**

Caution  
Handle as potentially biohazardous material under at least Biosafety Level 1 containment. This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

General Media Requirements  
**Suspension or adherent culture:** Use CD CHO Medium supplemented with 8 mM L-Glutamine and 10 mL/L of HT Supplement. Protect medium from light.

### Note:  
Antibiotics are not recommended; however, 5 mL/L of Penicillin-Streptomycin may be used when required.

Thawing Cells  
Store frozen CHO-S cells in liquid nitrogen (vapor phase) until ready to use. Frozen cells are supplied in and may be thawed directly into CD CHO Medium. Use the following procedure to thaw cells.

1. Rapidly thaw frozen vial in a 37°C water bath. Triturate and transfer the entire contents of the cryovial into a 125 mL shake flask containing 27 mL of pre-warmed CD CHO Medium supplemented with 8 mM L-Glutamine and 10 mL/L of HT Supplement, and incubate in a 37°C incubator containing a humidified atmosphere of 5 to 10% CO₂ in air on an orbital shaker platform rotating at 120-130 rpm. Loosen caps of flasks to allow oxygenation/aeration.

2. Once the culture has reached >2 x 10⁶ viable cells/mL, determine viability using the trypan blue exclusion method.

3. Expand CHO-S cultures by seeding shake flasks at 1 to 2 x 10⁵ viable cells/mL or adherent cultures at 1 to 2 x 10⁵ cells/cm² by diluting cells in pre-warmed growth medium. See **Subculturing Cells** to maintain and subculture CHO-S cells in suspension or adherent culture.

### Note:  
We recommend subculturing cells for a minimum of 3 passages before use in other applications or transfer into serum-supplemented media.

Determining Cell Density and Viability  
Follow the procedure below to determine viable and total cell counts.

1. Transfer a small aliquot of the cell suspension to a microcentrifuge tube.

2. Determine viability using the trypan blue exclusion method.

3. Determine cell density electronically using a Coulter Counter or manually using a hemocytometer chamber.

Subculturing Cells  
Use the following procedures and recommended conditions to subculture CHO-S in suspension or adherent culture.

#### Subculturing Procedure: Suspension Cultures

1. Determine viable and total cell counts (see procedure).

2. Seed cells at the recommended density (see table), diluting in pre-warmed growth medium. Put flasks in incubator with caps loosened to allow for oxygenation/aeration.

### Subculturing Procedure: Adherent Cultures

Use this procedure to subculture CHO-S cells grown in a T-75 cm² flask. If you are using other-sized flasks, scale the reagent volumes up or down accordingly.

1. Remove media from the flask. Rinse the flask with 5 mL of Dulbecco’s Phosphate-Buffered Saline (D-PBS) without Ca²⁺ or Mg²⁺ and remove.

2. Add 1 mL of pre-warmed 0.25% Trypsin-EDTA to the flask, and incubate until the cells have detached (about 2 to 5 minutes at room temperature).

3. Add 9 mL of serum supplemented growth medium to the flask and transfer the cell suspension to a 15 mL centrifuge tube.

4. Determine viable and total cell counts (see procedure).

5. Seed cells at the recommended density (see table), diluting in pre-warmed growth medium. Put flasks in incubator with caps loosened to allow for oxygenation/aeration.

#### Recommended Conditions

<table>
<thead>
<tr>
<th>Cell density</th>
<th>Suspension Cultures</th>
<th>Adherent Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;2 x 10⁶ viable cells/mL</td>
<td></td>
<td>T-75 cm² to T-162 cm²</td>
</tr>
<tr>
<td>Culture vessel</td>
<td>125 or 250 mL poly-carbonate disposable sterile Erlenmeyer flask containing 30-40 mL or 75-100 mL total working volume of cell suspension, respectively</td>
<td>disposable sterile T-flasks. Dilute cells in a total working volume of 15-20 mL for T-75 cm² flasks and 40-50 mL for T-162 cm² flasks</td>
</tr>
<tr>
<td>Seeding density</td>
<td>1 to 2 x 10⁵ viable cells/mL</td>
<td>1 to 2 x 10⁵ viable cells/cm²</td>
</tr>
<tr>
<td>Incubation conditions</td>
<td>37°C incubator with a humidified atmosphere of 5 to 10% CO₂ in air on an orbital shaker platform rotating at 120-130 rpm; loosen caps to allow for oxygenation/aeration</td>
<td>37°C incubator with a humidified atmosphere of 5 to 10% CO₂ in air; loosen caps to allow for oxygenation/aeration</td>
</tr>
</tbody>
</table>

#### Scaling-Up CHO-S Cells

You may scale-up the CHO-S cultures in spinner flasks or stirred tank bioreactors using the guidelines below. Note that the appropriate spinner or impeller speed and seeding density should be determined and optimized for each system.

- **Spinner or impeller speed:** Determine the optimum spinner speed and impeller speed for your bioreactor depending on your needs.

- **Seeding density:** We use optimized seeding densities of 1 to 2 x 10⁵ viable cells/mL. **Note:** If the split ratio of cells to fresh media is <1:2, we recommend spinning down the cell suspension for 5 to 10 minutes at 100 x g, and resuspending the cell pellet in fresh CD CHO Medium prior to inoculating the spinner or bioreactor culture.
Freezing Cells

**Recommended Conditions**
- Freeze cells at a density of ≥1 x 10^7 viable cells/mL.
- Use a freezing medium composed of 50% fresh growth medium and 50% conditioned growth medium (day 2 to 4 cell conditioned media collected from CHO-S cultures during subculture procedure) and DMSO to a final concentration of 7.5%. Prepare freezing medium immediately before use. Filter-sterilize the freezing medium and chill at 4°C until use. Discard any remaining freezing medium after use.

**Freezing Procedure**
1. Grow the desired quantity of CHO-S cells in shake or T-flasks, harvesting when the cell density reaches ≥2 x 10^9 viable cells/mL.
2. Determine viable and total cell counts (see procedure on the previous page) and calculate the volume of freezing medium required to yield a final cell density of ≥1x10^7 viable cells/mL.
3. Prepare the required volume of freezing medium (see above).
4. Centrifuge cells from cell suspension (Step 1) at 100 x g for 5 to 10 minutes. Aseptically decant supernatant and resuspend the cell pellet in the pre-determined volume of chilled freezing medium.
5. Dispense aliquots of this suspension (frequently mixing to maintain a homogeneous cell suspension) into cryovials according to manufacturer’s specifications (i.e. 1.5 mL in a 2 mL cryovial).
6. Freeze cells in an automated, controlled-rate freezing apparatus or using a manual method following standard procedures. For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.
7. Transfer frozen vials to liquid nitrogen (vapor phase) storage.

**Note:** You may check the viability and recovery of frozen cells 24 hours after storing vials in liquid nitrogen by following the procedure outlined in Thawing Cells.

**Transfection**

General guidelines are provided below to transfect CHO-S cells using LIPOFECTAMINE™ 2000 or DMRIE-C Reagent available from Invitrogen. Refer to the manual accompanying each product for instructions. Other transfection reagents are suitable.

**Suspension cultures:** For optimal results, we recommend using LIPOFECTAMINE™ 2000 or DMRIE-C Reagent for transfection. If you use either LIPOFECTAMINE 2000 or DMRIE-C Reagent, you may transfect CHO-S cells directly in CD CHO Medium.

**Adherent cultures:** For optimal results, we recommend using LIPOFECTAMINE™ 2000 for transfection. If you use LIPOFECTAMINE 2000, transfer cells from monolayer culture in CD CHO Medium to serum-supplemented media (i.e., Dulbecco’s Modified Eagle Medium supplemented with 0.1 mM MEM Non-Essential Amino Acids and 10% Fetal Bovine Serum) several days prior to transfection. Cells should adapt directly into the serum-supple-mented media without any trouble. After transfection and selection, CHO-S cells can be expanded and re-adapted back into serum-free suspension culture in CD CHO Medium supplemented with 8 mM L-Glutamine, 10 mM HT Supplement, and the appropriate selective antibiotic.

**General Information**
Chinese Hamster Ovary (CHO) cells are among the most commonly used cell lines for transfection, expression and large-scale production of recombinant proteins. The CHO-S cell line is a stable aneuploid cell line established from the ovary of an adult Chinese hamster. The cell line has been distinguished as a separate sub-clone from the common CHO K1 cell line, and its history and stability have been extensively described.

The CHO-S cell line exhibits the following general features:
- Prepared from low passage Master Cell Bank cultures derived from parental CHO-S cells that were re-cloned by limiting dilution in CD CHO Medium, and selected for their superior serum-free cell growth and transfection efficiencies. The clonal derived cultures are maintained in serum-free conditions for only 20 to 25 total passages.
- Adapted to serum-free suspension growth in CD CHO Medium, a serum-free, protein-free, and chemically defined medium, formulated with no components of animal or human origin. **Note:** Cells also grow well in traditional media supplemented with serum.

**Product Qualification**

Frozen catalog CHO-S cells are performance tested for viability and cell growth post-recovery from cryopreservation, and are screened for mycoplasma and sterility. Master Cell Banks are screened for viruses, mycoplasma, and sterility. Species identity is confirmed by isozyme and karyotype analysis.

**References**

**Related Products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. No.</th>
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</thead>
<tbody>
<tr>
<td>CD CHO Medium</td>
<td>1 L</td>
<td>10743-029</td>
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<tr>
<td>200 mM L-Glutamine</td>
<td>100 mL</td>
<td>25030-081</td>
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<td>HT Supplement</td>
<td>50 mL</td>
<td>11067-030</td>
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<tr>
<td>Penicillin-Streptomycin</td>
<td>100 mL</td>
<td>15070-063</td>
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<tr>
<td>Dulbecco’s Phosphate-Buffered Saline (D-PBS)</td>
<td>500 mL</td>
<td>14190-144</td>
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<tr>
<td>Trypsin-EDTA</td>
<td>100 mL</td>
<td>25200-056</td>
</tr>
<tr>
<td>Dulbecco’s Modified Eagle Medium</td>
<td>500 mL</td>
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</tr>
<tr>
<td>10 mM MEM Non-Essential Amino Acids</td>
<td>100 mL</td>
<td>11140-050</td>
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<tr>
<td>Fetal Bovine Serum</td>
<td>500 mL</td>
<td>26140-079</td>
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<tr>
<td>DMRIE-C Reagent</td>
<td>1 mL</td>
<td>10459-014</td>
</tr>
<tr>
<td>LIPOFECTAMINE 2000</td>
<td>0.75 mL</td>
<td>11668-027</td>
</tr>
</tbody>
</table>

**Note:** Other reagent sizes are available.

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- **United States Technical Service:** 1 800 955 6288
- **Canada Technical Service:** 1 800 757 8257

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You may also contact your Invitrogen Sales Representative or visit our World Wide Web site at www.invitrogen.com.

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**CAUTION:** Not intended for human or animal diagnostic or therapeutic uses.