### General Procedure:
Fetal Bovine Serum, ES Cell Qualified is added to Mouse ES Basal Cell Media to a final concentration of 10 to 15%.

**Thawing**
Remove serum from frozen storage and place the bottle(s) overnight in a refrigerator at 2 to 8°C. When ready for use, transfer the bottle(s) to a 37°C water bath. Gently agitate the bottle(s) periodically in order to mix the solutes that tend to concentrate at the bottom of the bottle. Do not keep serum at 37°C any longer than necessary for it to thaw completely.

Alternatively, serum bottles may be placed in a 37°C water bath directly from frozen storage. Bottles should be agitated by a gentle swirling motion to enhance mixing and thawing.

Do not keep serum at 37°C any longer than necessary to thaw completely.

**Do not thaw serum at temperatures above 37°C.**

If you are not using all of your thawed serum at once, dispense it into single-use aliquots and store these at -70°C or -20°C. Protect the aliquots from light as much as possible during storage.

**NOTE:** Turbidity and flocculent material may be present after thawing. ATCC’s experience indicates that neither of these changes affect the performance of serum. If the presence of flocculent material or turbidity is a concern, they can be removed by aseptic filtration through a sterile 0.45 µm filter.

**Heat-Inactivation of Serum**

**CAUTION:** Heat-inactivation of serum is usually unnecessary and can be detrimental to the growth of some cells. We strongly recommend that you heat-inactivate fetal bovine serum only if it is required for a particular cell line.

1. Thaw serum following the directions above.
2. Preheat a water bath to 56°C with sufficient water to rise above the level of the serum in the bottle.
3. Mix thawed serum by gently swirling the bottle and then place the bottle in the 56°C water bath. (The temperature of the water bath will decrease.)
4. When the temperature of the water bath reaches 56°C again, heat the serum for an additional 30 minutes. Mix gently every five minutes to ensure uniform heating.
5. At the end of 30 minutes, remove serum from the water bath, cool, and store at -70°C or -20°C (see Storage, above).

**Quality Control Specifications**
Sterility tests are performed on each lot of fetal bovine serum using current USP and EP methods. Specific tests for contaminants are listed below in the Specification Certificate. This serum is tested for ability to support the growth and maintenance of embryonic stem cells in an undifferentiated state. Undifferentiated embryonic stem cells are determined by colony morphology and expression of three pluripotent markers. The results are compared to a control lot of serum. The serum is triple filtered through 0.1 µM filters and each lot of fetal bovine serum is tested for sterility. Fetal bovine serum is manufactured from fetal bovine blood collected in USDA inspected abattoirs located in the United States.

**Origin**
This fetal bovine serum is manufactured from fetal bovine blood collected in USDA-inspected abattoirs located in the United States.

<table>
<thead>
<tr>
<th>Test*</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterility Testing (Current USP and EP)</td>
<td>No growth</td>
</tr>
<tr>
<td>Bacteria and Fungi</td>
<td></td>
</tr>
<tr>
<td>Virus Testing (9 CFR 113.53)</td>
<td>Not detected</td>
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<tr>
<td>Fluorescent antibody</td>
<td></td>
</tr>
<tr>
<td>Bluetongue</td>
<td></td>
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<tr>
<td>Bovine Adenovirus</td>
<td></td>
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</tbody>
</table>
**Fetal Bovine Serum, ES Cell Qualified (ATCC® SCRR-30-2020™)**

**Storage Temp.**

Store fetal bovine serum at -70°C. If storage at -70°C is not possible, then store at -20°C. Do not store fetal bovine serum at temperatures above -20°C for any length of time. Avoid repeated freeze-thaws by dispensing and storing fetal bovine serum in aliquots. Fetal bovine serum is supplied in PETG plastic bottles.

**Mycoplasma Testing**

- **Pass**

- Large volume, direct culture
- Hoechst DNA stain

**Endotoxin (Limulus Amebocyte Lysate - Gel Clot)**

- ≤ 10 EU/mL

**Hemoglobin (Spectrophotometric)**

- Report result

**Growth Promotion**

- Pass

**ES Cell Testing**

- Pass

*Please consult the Certificate of Analysis for lot-specific test results.

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**ATCC Warranty**

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

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**Disclaimers**

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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