Please read this FIRST

**Storage Temp.**
- liquid nitrogen
- vapor phase

**Biosafety Level**
1

### Intended Use
This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

### Complete Growth Medium
Grow ES cells in Mouse ES Cell Basal Medium (ATCC SCRR-2011) that has been supplemented with the following components:
1. 0.1 mM 2-mercaptoethanol (Life Technologies Cat. No. 21985-023)
2. 1,000 U/mL mouse leukemia inhibitory factor (LIF) (Millipore Cat. No. ESG1107)
3. 10% to 15% ES-Cell Qualified FBS (ATCC® SCRR-30-2020) or an ES cell qualified serum replacement

Complete Growth Medium for Mouse ES Cells is stable for 14 days when stored at 2°C to 8°C. Grow ES cells in Mouse ES Cell Basal Medium (ATCC SCRR-2011) that has been supplemented with the following components:
0.1 mM 2-mercaptoethanol (Invitrogen Cat. No. 21985) 1,000 U/mL mouse leukemia inhibitory factor (LIF) (EMD Millipore Cat. No. ESG1107)

15% FBS, ES Cell Qualified (ATCC SCRR-30-2020) Complete Growth Medium for Mouse ES Cells is stable for 14 days when stored at 2°C to 8°C. This medium is formulated for use with a 5% CO2 in air atmosphere.

### Citation of Strain
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: RW.4 (ATCC® SCRC-1018™)

### Unpacking & Storage Instructions
1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

### Handling Procedure for Frozen Cells
**Complete Medium for Feeder Cells**
Feeder cells may be grown in medium containing fewer growth factors than those required by the ES cells. Feeder cells are available from ATCC. Consult the product sheet provided for the feeder cells you wish to use for medium requirements.

**Feeder cells should be initiated 24-48 hours prior to inoculating with embryonic stem (ES) cells.**

**Feeder Cells**
ATCC recommends culturing RW.4 on mouse embryonic fibroblasts (MEFs) that have been mitotically arrested by either irradiation or treatment with Mitomycin-C. RW.4 cells have been cultured on mitotically arrested MEF (CF-1) (ATCC® SCRC-1040™).

1. At least one day before plating the ES cells, prepare the desired combination of flasks with feeder cells to accommodate an initial ES cell seeding density of 30,000 cells/cm² to 50,000 cells/cm².
   *Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from this lot of ATCC® SCRC-1018™.*

2. Plate mitotically arrested mouse embryonic fibroblasts (MEFs) as a feeder layer at approximately 55,000 feeder cells/cm² in complete medium for feeder cells.

3. Refer to the product sheet for mitotically arrested MEF for detailed handling instructions.

**Feeder cells should be used within one week of plating. It is best to use feeder cells within 24-48 hours of initiation.**

**Embryonic Stem (ES) Cells**

1. **30 Minutes Prior to Handling Cells** – Pre-warm complete growth medium for ES cells at 37°C for at least 30 minutes before adding to cells.
2. **One Hour Prior to Thawing the ES Cells** – Perform a 100% medium change for the MEFs using complete growth medium for ES cells.
3. Thaw the vial of ES cells by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 90 seconds).
4. Remove the vial from the water bath before the contents are completely thawed, and decontaminate by dipping in or spraying with 70% ethanol.

*All of the operations from this point on should be carried out under strict aseptic conditions.*

5. Transfer the vial's contents plus 5 mL of complete growth medium for ES cells to a 15 mL centrifuge tube. Use an additional 1 mL of media to rinse the vial and transfer the liquid to the 15 mL tube. Add 4 mL of complete growth medium for ES cells to bring the total volume to 10 mL.
6. Spin the cells at 270 x g for 5 min. Aspirate the supernatant and resuspend the pellet in 2 mL of
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Storage Temp.
liquid nitrogen
vapor phase

Biosafety Level: 1

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3. 15% FBS, ES Cell Qualified (ATCC SCRR-30-2020)

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ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be
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Additional information on this culture is available on the ATCC web site at www.atcc.org