

# Certificate of Analysis

**Strain ICR Mouse Embryonic  
Fibroblasts (Irradiated)**

Catalog No. MUIEF-01002-10  
Lot Number: 110726H01

Cryopreservation Date: 2011-7-26  
Passage Number: 1

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## Viability

Cells are assayed for viability post-thaw using vital staining assay with trypan blue.  
Specification: Cells should exhibit  $\geq 80\%$  viability.

## Sterility

Bacterial and Fungal Contamination: Samples are inoculated and cultured in blood agar plate, thioglycolate broth, tryptocase soy broth and sabouraud dextrose agar.  
Specification: No growth must be observed.

Mycoplasma: Samples are tested for mycoplasma contamination using a PCR-based assay and direct culture.  
Specification: Results must be negative.

Endotoxin: Samples are tested for endotoxin contamination with LAL test.  
Specification: Results must show  $\leq 25\text{EU/ml}$ .

## Supporting Capability

Cells are shown to be able to support the growth of mouse embryonic stem (ES) cells and human ES and for the maintenance of the cells in undifferentiated state. The cells are used to culture mouse ESC and human ESC (in a separate set of plates) for three passages and each set of ESC are assayed for expression of cell-specific markers to confirm that cells remain undifferentiated. Immunostaining is performed using fluorochrome-conjugated antibodies specific to Oct4, SSEA-1, Nanog, SSEA-3, and SSEA-4.

Specification: For mouse ESC, the results indicate that  $\geq 90\%$  of colonies in a plate, and  $> 90\%$  of cells in each colony are positive for Oct4, SSEA-1, and Nanog, while  $\leq 5\%$  of colonies in a plate, and  $< 5\%$  of cells in each colony are positive for SSEA-3 and SSEA-4. For human ESC, the results must indicate that  $\geq 90\%$  of colonies in a plate, and  $> 90\%$  of cells in each colony are positive for Oct4, SSEA-3, SSEA-4, and Nanog, while  $\leq 5\%$  of colonies in a plate, and  $< 5\%$  of cells in each colony are positive for SSEA-1.

**Verification of Growth Arrest**

Cells are arrested with  $\gamma$ -ray. Cells are assayed after cryopreservation and confirmed to be mitotically inactivated. Cells are thawed and plated at low density ( $1.0\text{--}1.5 \times 10^4$  cells/cm<sup>2</sup>) and monitored for cell growth for 14 days. After 14 days, the results indicate that no growth of cells is observed.

**Results:**

Meet all specifications

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