

## Certificate of Analysis

**Description: OriCell™ KO-Certified 3 Drug-Resistant Mouse Embryonic Fibroblasts, Irradiated**

**Cryopreservation Date:** 01-24-2013

**Passage Number:** 1

**Cat #:** MUDEF-01002

**Lot #:** 130124E31

Test Parameters	Specification	Result
<b>Viability<sup>1</sup></b>	Cells must exhibit $\geq 80\%$ post-cryopreservation viability	Pass
<b>Sterility<sup>2</sup></b>	No growth must be observed	Pass
<b>Mycoplasma<sup>3</sup></b>	No growth must be observed	Pass
<b>Endotoxin<sup>4</sup></b>	$\leq 25$ EU/ml	Pass
<b>Stem Cell Support<sup>5</sup></b>	ESCs grown on cells must show $\geq 90\%$ positivity for expression of stem-cell specific markers including SSEA-1, Nanog and Oct4 and $\leq 5\%$ positivity for SSEA-3 and SSEA-4.	Pass
<b>Growth Arrest<sup>6</sup></b>	No cell growth must be observed	Pass
<b>Knockout Certified<sup>7</sup></b>	Cells are used to generate 6 in-house knockout mouse embryonic stem cell lines that give rise to chimeras and germline transmission animals	Pass

**Notes:**

- Cells are assayed for viability post-thaw using vital staining assay with trypan blue.
- Bacterial and Fungal Contamination: Samples are inoculated and cultured in blood agar plate, thioglycolate broth, tryptocase soy broth and sabouraud dextrose agar.
- Samples are tested for mycoplasma contamination using a PCR-based assay and direct culture.
- Samples are tested for endotoxin contamination with LAL test.
- Cells are assayed for supporting the stemness of ESCs in culture by immunostaining with fluorochrome-conjugated antibodies against stem cell-specific surface antigens including SSEA-1, Nanog, Oct4, SSEA-3 and SSEA-4.
- $\gamma$ -irradiated cells are plated at low density ( $1.0-1.5 \times 10^4$  cells/cm<sup>2</sup>) post-thaw and monitored for cell growth for 14 days by hemocytometer cell counting. Cell density is calculated as an indicator of cell proliferation.
- Cells are validated for their use as feeder cells in creating in-house knockout or knockin mouse ESCs that give rise to germline transmission knockout or knockin mice.

*Jane Chen*

Jane Chen  
Quality Assurance Manager  
Mar 22, 2013

