

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
Viability¹	Cells must exhibit $\geq 80\%$ post-cryopreservation viability	Pass
Sterility²	No growth must be observed	Pass
Mycoplasma³	No growth must be observed	Pass
Endotoxin⁴	≤ 25 EU/ml	Pass
Stem Cell Support⁵	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
Growth Arrest⁶	No cell growth must be observed	Pass
Knockout Certified⁷	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.
- γ -irradiated cells are plated at low density ($1.0-1.5 \times 10^4$ cells/cm²) 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.

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Mar 22, 2013

