

# Certificate of Analysis

**Strain 129 Mouse Embryonic Stem Cells**

Catalog No. MUAES-01001

Lot Number: 120621E01

Cryopreservation Date: 2012-6-21

Passage Number: 20

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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 on 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 a concentration of  $\leq 25\text{EU/ml}$ .

## Karyotype

Cells are analyzed for karyotype by performing  $\geq 20$  metaphase spreads.

Specification: Results must be indicated that the cells possess 40 chromosomes (20 diploid pairs) plus 2 sex chromosomes (X, Y). The profile must match the published profile of Strain 129 mouse with no gross abnormalities.

## Verification of Undifferentiated State

Cells are analyzed for expression of cell-specific markers after cryopreservation. Cells are immunostained with fluorochrome-conjugated antibodies specific to OCT-4, SSEA-1, Nanog, SSEA-3 and SSEA-4.

Specification: Cells must be shown to remain undifferentiated when cultured on mouse embryonic feeder cells after cryopreservation. Results must indicate that  $\geq 90\%$  of colonies in a plate and  $\geq 90\%$  of cells in each colony are positive for OCT-4, SSEA-1 and Nanog, while  $\leq 5\%$  of colonies in a plate and  $\leq 5\%$  of cells in each colony are positive for SSEA-3 and SSEA-4.

**Differentiation Potential**

Cells are assayed after cryopreservation for their ability to differentiate into embryoid bodies and express cell-specific markers indicative of the three germ layers.

Specification: Results must indicate positive expression of  $\alpha$ -3-tubulin or nestin (ectoderm-specific markers), smooth muscle actin (mesoderm-specific marker), and AFP (endoderm-specific marker).

**Results:**

All specifications have been met.

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