

Certificate of Analysis

**Strain 129 Mouse Embryonic Stem Cells
with GFP**

Cryopreservation Date: 2009-7-13

Passage Number: 23

Catalog No. MUAES-01101

Lot Number: 090713B02

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 ≥ 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.

GFP Expression

Expression of constitutive GFP is assayed by visual inspection of GFP fluorescence signal.

Specification: $\geq 80\%$ of the cells in each colony express GFP at high levels after 5 passages when cultured at normal proliferation conditions.

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 $\beta 3$ -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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Sep 2, 2009