

Certificate of Analysis

**Strain C57BL/6 Mouse Embryonic Stem Cells
with GFP**

Cryopreservation Date: 2009-7-10
Passage Number: 23

Catalog No. MUBES-01101
Lot Number:090710B02

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 $\leq 25\text{EU/ml}$.

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 C57BL/6 mouse with no gross abnormalities.

GFP Expression

Expression of constitutive GFP is assayed by visual inspection of GFP fluorescence signal.
Specification: $\geq 90\%$ 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 30, 2009