

Certificate of Analysis

Product Murine Embryonic Fibroblast Feeder Cells

Lot # MEF_SW
04/24/03

Description Murine Embryonic Fibroblast isolated from Swiss Webster Mice (Taconic Model Number SW) and mitotically inactivated by Mitomycin C treatment

Model # MEF_SW

Unit 2 cryovials containing 1 ml each

Concentration 6.48 x 10⁶ Cells per ml

Average Percent Viability for lot 93.4%

Plating Efficiency 25%

Cell Characteristics

Mycoplasma Test
M-250 Multi-media Direct Culture and DNA Fluorochrome Assay (Mycoplasma) Testing. Test results received 5/20/03. Negative – A reaction with staining limited to the nuclear region, which indicates no mycoplasma contamination.
M-100 DNA Fluorochrome Cell Shipper Assay (Mycoplasma) Testing. Test results received 5/30/03. Negative – A reaction with staining limited to the nuclear region, which indicates no mycoplasma contamination. Tested in compliance with GMP standard at Bionique Testing Laboratories, Inc. (Saranac Lake, NY).

MAP Test with LCM Challenge
MAP (Mouse Antibody Production) Test results received 7/25/03. Negative. No significant titers to indigenous murine viruses were detected and all mice survived the challenge for LCM virus. Tested in compliance with GLP guidelines at now Taconic-Anmed (Rockville, MD).

Shipping conditions Cells shipped frozen on dry ice.

Storage conditions -180°C

Operations _____

Date _____

Quality Assurance _____

Date _____

Guidelines for preparing Murine Embryonic Fibroblast Feeder Cells for use

- To make feeder plates thaw a frozen vial of mitomycin C treated MEF cells quickly in a 37°C water bath. Prepare gelatinized plates before seeding fibroblasts.
- Add cells to 10 ml DMEM, 2mM L-glutamine, 10% FBS and 1% Penicillin-Streptomycin) and centrifuge at 270g for 5 min.
- Remove the supernatant and re-suspend cell pellet into an appropriate volume of growth media. Aliquot fibroblasts per the densities stated below.
- 1 vial can be thawed onto the following gelatinized plates:
 - 1 x P-100 (~6.0 x 10⁶ cells per plate)
 - 3 x P-60 (~2.0 x 10⁶ cells per plate)
 - 7-9 x P-35 (~ 7.0-8.0 x 10⁵ cells per plate)Incubate cells at 37°C, 5% CO₂.
- Feeder layers should form a confluent monolayer and the surface of the plate should be evenly and completely covered with fibroblasts. Allow feeders to attach preferably overnight before adding ES cells. Gelatinized plates can be used immediately for culturing ES cells.
- Change the medium to ES cell medium prior to adding ES cells.

Michael P. Mafise, Wojtek Auerbach and Alexandra L. Joyner (2000). **Gene targeting: a practical approach**. Protocol excerpted from Chapter 3, Production of targeted embryonic stem cell clones. Alexandra L. Joyner (ed.), 2nd edition, *Oxford University Press, Oxford, England*.