ATCC

Product Sheet

# Mitomycin C (ATCC® SCRR-3020™)

**Please read this FIRST**

Storage Temp.
**When stored at 2°C to 8°C, the product is stable until the expiration date on the label.**

Biosafety Level
**1**

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## Description

**Product Description:**
Mitomycin C
**CAS Number:** 50-07-7
**Molecular Weight:** 334.3
**Molecular Formula:** $C_{15}H_{18}N_4O_5$

Embryonic stem cells are best supported in vitro by growth on mitotically arrested feeder cells. Mitotic inactivation, by treatment of mouse embryonic fibroblasts (MEFs) and other feeder cell cultures with Mitomycin C, prevents division of the fibroblasts while allowing the cells to survive and produce key proteins such as Leukemia Inhibitory Factor (LIF) that support the self renewal cycle of the stem cells.

Mitotic inactivation can be achieved by irradiation, but most researchers choose to avoid the cost and safety issues involved with use of a gamma-irradiation source in the laboratory. Alternatively, treatment with Mitomycin C is fast, convenient and does not require the use of sophisticated equipment. However, the use of Mitomycin C still requires the implementation of stringent safety measures during use, since the compound is toxic to both cultured cells and to humans.

Mitomycin C is an antineoplastic antibiotic that inhibits DNA synthesis by cross-linking DNA at 5' -CpG- 3' sequences. It produces oxygen radicals and is preferentially toxic to hypoxic cells. Care must be taken when preparing the Mitomycin C solution. The fibroblasts must be extensively washed following treatment to prevent toxic effects to embryonic stem (ES) cells, or other cell types that require the use of a feeder cell layer for propagation.
**Volume:** 2 mg

## Directions for Use

**Preparation of the Mitomycin C Solution**

1. Bring the vial of Mitomycin C to room temperature.
2. Prepare a 5% DMSO solution in PBS without $Ca^{2+}$ and $Mg^{2+}$ (95:5 PBS-DMSO Solution) by combining 4.75 mL PBS with 0.25 mL DMSO. Keep all solutions sterile throughout preparation.
3. Using a 5 mL syringe and an 18 ga. needle, inject 4 mL of the 95:5 PBS-DMSO Solution into the vial containing the 2 mg of dry Mitomycin C powder. Insert a second needle to vent the vial. Mix to dissolve and draw the solution back into the syringe. Transfer to a 5 or 15 mL sterile tube. (Filter sterilize through a 0.22 µm pore filter if aseptic conditions were not strictly maintained.)
4. Concentration of the prepared Mitomycin C stock solution is 0.5 mg/mL.
5. Use the prepared Mitomycin C stock solution immediately, or store, protected from light, for up to 2 weeks at 2°C to 8°C.
6. The final concentration for use is 10 µg/mL (dilute the Mitomycin C stock solution 1:50 in MEF Complete Growth Medium).

**Treatment of MEFs with Mitomycin C**
**Note:** This protocol is written for MEFs grown in T-75 flasks.

1. Remove existing medium from 90% confluent mouse embryonic fibroblast (MEF) cells.
2. Replace with 10 mL fresh Complete MEF Growth Medium containing 10 µg/mL Mitomycin C (e.g., 200 µL Mitomycin C solution per 10 mL of Complete MEF Growth Medium).
3. Incubate the flask(s) at 37°C with 5% $CO_2$ for 2 to 3 hours (minimum time is 2 hours).
4. Remove the medium containing Mitomycin C and transfer it to a waste bottle. Make sure that the waste bottle is clearly labeled as Mitomycin C waste to facilitate proper disposal later.
5. Wash the cells once with 10 mL of 1x PBS per T-75 flask. Remove the liquid and add it to the waste bottle from step #4, above.
6. Wash each T-75 flask one more time with 10 mL of 1x PBS. Transfer the wash PBS to the waste bottle from step #4, above.

**Trypsinization of Treated MEFs**

1. Add 3 to 5 mL of 0.25% trypsin/0.53 mM EDTA solution to each flask and swirl to ensure the entire cell surface is covered.
2. Incubate at 37°C for 3 to 5 minutes. Lightly tap the sides of each flask/dish to help detach cells from the surface.
3. Add an equal volume of Complete MEF Growth Medium (containing serum) to each flask to inactivate the trypsin.
4. Pipette the cell suspension in each flask/dish up and down multiple times in order to form a single-cell suspension.
5. Collect all cell suspensions in one or more 50 mL tubes.
6. Rinse each flask once more with 5 mL of medium and add the additional volume to the tubes in step #5, above.

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7. Spin down the cells 1100 rpm (270 x g) for 5 minutes at room temperature.
8. Carefully remove the supernatant without touching the cell pellet.
9. Resuspend the cell pellet in 5 mL to 10 mL Complete MEF Growth Medium.
10. Count the cells to determine the number of viable cells available for plating new vessels or for cryopreservation for later use. Cryopreserved treated MEFs should be frozen at high densities (e.g., 6 x $10^6$ cells/mL) to allow for some cell loss following freeze/thaw.

**Plating Mitomycin-Treated MEFs**

**Note:** Tissue culture vessels may be coated with 0.1% gelatin solution (PCS-999-027) to improve adherence of the MEFs to the vessel surface.

- Plate the treated MEF cells directly onto tissue culture vessels (plates, dishes, or flasks).

**Plating Guidelines:**

1. Mitotically arrested MEF feeder cells should be plated at a density of 4.0 x $10^4$ cells/$cm^2$ to 8.0 x $10^4$ cells/$cm^2$ (depending on cell sizes of feeder cells). However, when receiving ES cells, the supplier's recommendations should always be followed.
2. Place the vessels in a 37°C incubator with 5% $CO_2$. MEFs will attach within 20 minutes and spread out within 12 to 24 hours.
3. Before plating ES cells on the MEF feeder layer check the vessels and change the medium to the appropriate ES cell culture medium.

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