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Product Information

SMAC.4M TagRFP Smooth Muscle Cells (derived from mouse embryonic stem cells) 2M Kit with Puromycin and Medium

Catalog Number **AXIO0053**

TECHNICAL BULLETIN

Product Description

SMAC.4M TagRFP Smooth Muscle Cells 2M are derived from transgenic mouse embryonic stem (mES) cells. These cells carry a puromycin resistance gene for chemical selection and a red fluorescent protein (TagRFP) reporter gene. Both genes are under the control of the smooth muscle cell-specific *Acta2* promoter.

SMAC.4M cells are produced by *in vitro* differentiation of mouse embryonic stem (ES) cells and puromycin selection. Stimulation by angiotension II, bradykinin, endothelin 1, or carbachol results in intracellular calcium mobilization and induces slow contractions of the cells.

Components

SMAC.4M TagRFP Smooth Muscle Cells 1 vial
(derived from mouse embryonic stem cells) 2M
(SMAC.4M TagRFP-eS-2M)
2×10^6 mouse ES cell derived cells
Catalog Number AXIO0034

- SMAC.4M Smooth Muscle Culture Medium 250 mL
(SMAC.4M Culture Medium)
Catalog Number AXIO0075

- Puromycin Solution 50 µL
Catalog Number AXIO0078
Used when the cells are cultured for >1 week.

Reagents and Equipment Required but Not Provided.

- Collagen I solution, Catalog Number C3867, for coating cell culture vessels (~4 mg/mL, concentration varies from lot-to-lot). Alternatively, use BD BioCoat™ plates pre-coated with collagen I.
- 0.4% Trypan Blue Solution, Catalog Number T8154
- Glacial acetic acid for dilution of Collagen I solution
- Syringe with sterile filter (sterile Millex® syringe filter, Millipore)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Preparation of Collagen I-coated cultureware

1. Prepare 20 mM acetic acid solution by adding 115 µL of glacial acetic acid to 100 mL of ultrapure water. Pass through a syringe sterile filter.
2. Dilute Collagen I solution, Catalog Number C3867, to 50 µg/mL with 20 mM acetic acid solution.
3. Coat vessels with diluted Collagen I solution (50 µg/mL, 0.2 mL/cm^2) and incubate at room temperature for one hour.
4. Carefully aspirate remaining Collagen I solution.
5. Rinse twice with PBS solution to remove remaining acid.
6. Cultureware may be used immediately, or may be air dried and stored at 2–8 °C for up to one week under sterile conditions.

Preparation of Culture Medium with Puromycin

1. Thaw the vial of Puromycin Solution at room temperature, Catalog Number AXIO0078.
2. Add 0.1 µL of Puromycin Solution per mL of SMAC.4M Smooth Muscle Culture Medium to give 1 µg/mL.
3. Mix well, but avoid foaming, and store the Culture Medium with puromycin at 2–8 °C.

Storage/Stability
Store the smooth muscle cells (Catalog Number AXIO0034) at –196 °C.

AXIO0075 and AXIO0078 are shipped on dry ice and storage at –20 °C is recommended.

Procedure
SMAC.4M cells are genetically modified mouse cells and should be handled according to local directives (Typically Biosafety level 1).

The cells should be cultured using sterile cell culture techniques and good laboratory practices.

Cells can be inactivated by autoclaving at 121 °C for 20 minutes.

Thawing and Seeding of Smooth Muscle Cells

1. Thaw the SMAC.4M Smooth Muscle Culture Medium, Catalog Number AXIO0075, at 4 °C and mix carefully after thawing. Store the medium at 4 °C until use.
   Note: Avoid foaming when mixing the medium.

2. Pre-warm the required amount of Culture Medium in a 37 °C water bath.

3. Thaw the vial of SMAC.4M TagRFP-eS-2M cells in a 37 °C water bath until just a sliver of ice remains (~2 min in the water bath).

4. After thawing, immediately transfer the cell suspension into a 50 mL centrifuge tube filled with 8 mL of Culture Medium.

5. Rinse the vial with an additional 1 mL of Culture Medium to collect remaining cells and add to the cell suspension in the 50 mL tube (10 mL total volume in tube).

6. Spin the cell suspension at 200 × *g* (~1,000 rpm with most swinging bucket rotors) for 5 minutes.

7. Resuspend the cell pellet carefully in 2 mL of Culture Medium. Mix an aliquot (20 µL) of the cell suspension with an equal volume of 0.4% Trypan Blue Solution, Catalog Number T8154, for viability staining and incubate for 2 minutes at room temperature.

8. Count the cells in a Neubauer hemocytometer and calculate the number of viable cells. Keep in mind to correct calculated number for the dilution factor of 2 (mixed 1:1 with Trypan Blue Solution).

9. Seed the cells at a density of 1×10^5 viable cells per cm^2 (surface area) of cultureware (use 32,000 cells for each well of a 96 well plate).

10. Incubate the cells in a cell culture incubator at 37 °C with 7% CO_2 and 95% humidity. Cells are ready for functional experiments after 24–48 hours.

Maintenance of Smooth Muscle Cells

1. Change culture medium every 2–3 days, after inspection. If medium starts changing color, change the medium.

2. If cells are cultured for >1 week, it is recommended to use the prepared Culture Medium with Puromycin to prevent proliferation of unwanted cells.

Axiogenesis Label license

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This product is covered by patent families including, but not limited to, EP1348019; EP1002080; EP1745144; EP1644485; JP4904153; JP4159358; JP3956154; JP4814875; DE10136702 and other families of patent applications ("AXIOGENESIS Intellectual Property"). Purchase of the product does not transfer any rights other than those outlined below.

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