

TROPHOBLAST STEM CELL PROTOCOLS

The protocols and reagents described here are for the maintenance and derivation of trophoblast stem (TS) cell lines.

Original reference with some modifications:

Tanaka, S. Kunath, T, Hadjantonakis, A-K, Nagy, A and Rossant, J.
Promotion of Trophoblast Stem Cell Proliferation by FGF4.
Science (1998) 282, 2072-2075

Media Composition

Base TS medium

500 ml RPMI 1640 (without Glutamine) (Sigma)
130 ml fetal bovine serum (final concentration - 20%) (Life Technologies)
6.5 ml 100mM sodium pyruvate (final concentration - 1mM) (Life Technologies)
6.5 ml 10mM β -mercaptoethanol** (final concentration - 100 μ M) (Sigma)
6.5 ml 200mM L-glutamine (final concentration - 2mM) (Life Technologies)
antibiotics (pen/strep @ 50 μ g/ml each final concentration) if desired

** Stock solution is prepared by adding 70 μ l β -mercaptoethanol into 100mL of PSB and aliquot at 6.5mL. Aliquots can be stored at -20°C until used.

TS media is substituted daily with 1X FGF and 1X Heparin as described below;

1000x FGF4

- Human recombinant FGF4 R&D systems cat #235-F4-025, 25 μ g
- Re-suspend lyophilized FGF4 in its vial with 1.0ml of PBS/0.1% w/v fraction V BSA*.
Aliquot and freeze at -20°C.
- Thaw each aliquot as needed and store at 4°C; do not re-freeze.

1000x Heparin

- Heparin (Sigma cat # H3149 10,000 units)
- Re-suspend heparin in PBS to a final concentration of 1.0 mg/ml (1000X) and store at -20°C.
- Thaw aliquots as needed and store at 4°C.

Thawing TS cells

Hand-thaw the vial until the suspension is approximately half ice crystals and add contents to 4.0ml TS medium, and spin to pellet. Re-suspend the cells in 5ml complete TS medium (+ FGF and Heparin). Plate pellet into 1X 6 cm plate on E12.5 day MEFs which have been previously mitotically inactivated. Change medium the next day to get rid of non-adherent cells and continue to feed every two days until cells are ready for passage.

Culturing TS cells

Culture Conditions: Culture TS cells are cultured in a standard tissue culture incubator (37°C, 5% CO₂ incubator. The medium is normally changed every two days, and the cells are passaged (1:10 to 1:20) every 4 to 6 days, or when the culture has reached approximately 80% confluency. Passaging TS cells at higher densities (e.g., 1:3 or 1:5) may lead to precocious differentiation.

Passaging Cells: When cells reach 80% confluency, cells are passaged by trypsin into very small clumps with some single cells. A complete single-cell suspension is not required and it may even be detrimental to the culture. Trypsin with 0.25% trypsin for 3-4 minutes at 37°C with some pipetting up and down is usually sufficient.

TS Cell Matrix: TS cells may be grown up on either MEFs (A) or regular tissue culture plastic in the presence of feeder-conditioned media (B). Gelatin-coating is not required.

(A) Culturing TS cells on MEFs

Preparation of MEFs: One day prior to splitting cells plate mitomycin-treated embryonic 12.5 day MEFs (E12.5) at a density of 1.8×10^6 cells/per full 6 well plate.

Preparation of media: Media should be prepared daily by supplementing 10 ml TS medium with 10 μ l 1000x FGF4 stock and 10 μ l 1000x heparin stock.

(B) Culturing TS cell on tissue culture plastic

TS cells grow well on standard tissue culture dishes if the medium is supplemented with FCM. The dishes do NOT need to be gelatin-coated as mentioned in Tanaka et al.

Preparation of Feeder-Conditioned Medium: Feeder-conditioned medium (FCM) is used to culture TS cells in the absence of feeders (MEFs). Prepare mitomycin-treated primary embryonic fibroblasts (MEFs) in 15cm dishes at 6.0×10^6 cells/per plate. Add 35 mL of TS media (minus FGF and Heparin) per plate and harvest every 3 days; FCM can be harvested up to 3 times from each 15cm plate. Filter collected FCM to remove floating cells and debris with a 0.45 μ m filter unit, store at -20°C in 35 ml aliquots. Thaw each aliquot as needed and store it at 4°C; do not re-freeze.

Working Media Solution: Combine 7ml FCM medium with 3ml TS medium (none FCM media) supplement the total volume (10mL) with 10 μ l 1000x FGF4 stock and 10 μ l 1000x heparin stock. Culture the TS cells in the above medium; feed and passage the cells as above.

Removing MEFs from TS cell cultures

When switching from MEFs cells to feeder free conditions it may be desirable to get rid of the MEF cells immediately. The different adherence rates of MEFs cells (fast) and TS cells (slow) can be used to obtain a pure TS cell population.

Passage cells to a new plate and incubate the culture for 1.5 hr at 37°C in 5% CO₂. Remove the supernatant and plate onto another dish. This population of cells should consist almost entirely of TS cells. Since some TS cells do adhere along with the MEFs, the desired passage density may vary.

Freezing TS cells

Prepare 2x freezing medium (50% FBS, 30% TS medium, 20% DMSO), and cool to 4°C. Harvest and pellet TS cells as described above. Resuspend in TS medium and add an equal volume of 2x freezing medium. Freeze the cells slowly at -70°C overnight and then transfer to liquid nitrogen after 24 to 48 hours. A confluent 100 mm dish has enough cells for about 6 vials.

Updated: Aug 2015