

Materials List for:

Derivation of Mouse Trophoblast Stem Cells from Blastocysts

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URL: <https://www.jove.com/video/1964>

DOI: [doi:10.3791/1964](https://doi.org/10.3791/1964)

Materials

Reagents
<p>TS medium: RPMI-1640, 20% FBS, 1 mM sodium pyruvate, 100 mM beta-mercapt-ethanol and 100 mg/ml penicillin-streptomycin.</p>
<p>Mitomycin C: Dissolve 2 mg mitomycin C (Sigma M-0503) in 2 ml PBS and add this 2 ml mixture into 200 ml TS medium (10 ng/ml). Make 20 aliquots (10 ml) and store at -20°C until use. Add one aliquot per 100 mm plate.</p>
<p>Mouse embryonic fibroblast-condition medium (MEF-CM): Plate MEFs in 100 mm plates (1 x 10⁶/plate). Next day, add 10 ml TS medium per dish and continue to culture for 48 hours. Collect the culture medium, filter them (0.45 mm) and store at -20°C in 35 ml aliquots. Thaw each aliquot when needed which can be stored at 4°C. The MEFs can be used to prepare for two more batches of CM before they become confluent.</p>
<p>PBS/BSA: Dissolve BSA, fraction V (Sigma A3311, 0.1% (w/v)) in PBS (10 ml), filter through a 0.45 mm syringe filter and make 1 ml aliquots in 1.5 ml tubes. Store at -70°C and thaw one tube when needed to prepare FGF4.</p>
<p>FGF4 (1000x): Resuspend lyophilized FGF4 (Sigma F8424, 25 mg) with 1 ml of the PBS/BSA solution. Mix well and make 10 aliquots (100 ml) into 1.5 ml tubes and store at -70°C. Thaw each tube when needed and store the remaining at 4°C, do not re-freeze. Dilute 1000 times in TS medium to obtain 1x FGF4 (25 ng/ml).</p>
<p>Heparin (1000x): Resuspend heparin (Sigma H3149, 10,000 units) in PBS to a final concentration of 1 mg/ml (1000x). Make 100 ml aliquots into 1.5 ml tubes and store at -70°C. Thaw aliquots when needed and store the remaining at 4°C, do not re-freeze. Dilute 1000 time in TS medium to obtain 1x heparin (1 ng/ml).</p>