Culture Reagents for Mouse Keratinocyte Stem Cells

Collagen 1 from rat tail (Becton Dickinson BC 354236): Stock solution at 50 µg/ml in 0.02M acetic acid. Filter sterilise using 0.2micron filter.

Foetal Calf Serum-Gold (PAA A15-151)

Chelex 100 (Bio-Rad 354400)

FAD medium: DMEM/Ham's F12, 3.5:1.1, low calcium (0.05mM Ca^{2+}), (custom made by Biochrom, Berlin, Germany)

FAD Medium formulation: DMEM/Ham's F12, (3.5 : 1.1) with 0.05 mM Ca^{2+}, 10% FCS (FCS Gold, PAA), 0.18 mM adenine (Sigma A2786), 0.5 µg/ml hydrocortisone (Sigma H4001), 5 µg/ml insulin (Invitrogen 12585014), 10^{-10} M cholera toxin (Sigma C8052), 10 ng/ml EGF (Invitrogen 53003-018), 2 mM glutamine, 1mM pyruvate.

Foetal Calf Serum-Gold is Chelex-treated to remove Ca^{2+} ions: 20g of Chelex 100/500mls FCS-Gold is left to rotate overnight at 4℃, then pre-cleared by filter paper filtration, sterile filtered and stored in 50ml aliquots at -20℃.

Supplement stock solutions are prepared as follows under sterile conditions or sterile filtered after preparation and stored in aliquots at -20C unless otherwise indicated

- Adenine (250X): 45mM in 50mM HCl (300mg dissolved in 50mM HCl solution). Add 2ml of stock solution to 460mls FAD medium
- Hydrocortisone (500X): 250 µg/ml l in ethanol. Add 1 ml of stock solution to 460mls FAD medium.
- Insulin (800X): 4 mg/ml. Add 0.63ml of stock solution to 460mls FAD medium.
- EGF (10**00**X): 10 µg/ml in FAD medium. Add 0.5ml stock solution to 460mls FAD medium. (
- Cholera toxin (10^{-5} M): 1mg/1.18 ml sterile water, store at 4℃. Add 5µl of stock solution to 460mls FAD medium. (Alternatively, formulate Cholera toxin (10^{-6} M): i.e. 1mg/11.8 ml sterile water and add 50µl to 460ml FAD medium)

0.05% Trypsin/0.02% EDTA

0.02% EDTA in PBS

Keratinocyte freezing medium: 90% Chelex-treated FCS-Gold, 10 % dimethyl sulphoxide, (cell culture grade)