

TRAMP-C line Culture Media

Dulbecco's Modified Eagle Medium 1X high glucose w/ L-glutamine & w/o sodium pyruvate from Gibco (Cat. No. 11965-084).

5% Nu-Serum IV from Collaborative Biomedical Products (Cat. No. 55004).

5% FBS from Hyclone (Cat. No. A-1115).

5µg/ml insulin see below for stock.

25u/ml Penicillin-Streptomycin from Gibco at 10,000 units/ml (Cat. No. 15140 122). (1.25 ml stock/500ml of medium)

10⁻⁸ M DHT in 100% ETOH (Stock 10⁻⁴ M) Sigma (D-7149) culture grade or from Steraloids (5alpha-androstan-17beta-ol-3-one) Cat#(A-8500), phone (603)654-5230, steraloids@jlc.net

Cells are grown on tissue culture plastic.

Insulin Stock (2.5 mg/ml)

Insulin Sigma Cat. No. I-5500.

1. 12.5 mg insulin in 5 ml Ca⁺⁺, Mg⁺⁺ free Hanks.
2. Add 1N NaOH at 3µl drops until all of the insulin is in solution.
3. Membrane filter to sterilize.
4. Store frozen at -20 degree.
5. Use at final concentration of 5µg/ml of medium.

Splitting TRAMP-C lines

Plates are rinsed with Ca⁺⁺, Mg⁺⁺ free Hanks for ~1 minute. (Available from Gibco, Cat. No. 14170-112)

Plates are trypsinized using 0.25% trypsin, 1 mM EDTA·4Na in Ca⁺⁺, Mg⁺⁺ free HBSS. (Available from Gibco, Cat. No. 25200)

100mm plate ~1ml trypsin

35mm plate ~0.1ml trypsin

Watch plates and when the cells release after tapping or shaking the plate neutralize the trypsin with media containing 10% serum. Neutralize trypsin with 10 fold excess of serum containing media. Culture media is what I use.

Rinse plate with media.

Spin cells for 10 minutes.

Resuspend in culture media and plate on tissue culture plates.