Making E8 medium for ES/iPS culture

1. Dissolve 1.358g of sodium bicarbonate in 50ml water. Warm the water at 37C to completely dissolve the salt.
2. Dissolve 10mg of sodium selenite in 1ml water. Then dilute this solution 1:100 with water to make 100ug/ml solution.
3. Add 350ul of 100ug/ml sodium selenite solution to the 50ml sodium bicarbonate solution. Then filter sterilize.
4. Aliquot into 10ml aliquots. Each aliquot is enough for 500ml of DMEM/F12.
5. Dissolve 160mg of L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate (Sigma: A8960) in 5ml of water. Filter sterilize. Then aliquot into 1ml aliquot. Each aliquot is enough for 500ml DMEM/F12.
6. Freeze all aliquots at -80C.
7. To make E4 media. Add 10ml of sodium bicarbonate and sodium selenite solution and 1ml of VitC solution from above to 500ml of DMEM/F12.
8. E4 media made this way should have osmolarity around 310 and pH around 7.4.
9. To make E6 media, add to the above E4 medium the following: 0.25ml insulin solution (10mg/ml) and 0.5ml transferrin (10mg/ml). Mix well. (Note: The original E8 formula contains 20ug/ml inuslin. This recipe contains 5ug/ml insulin. Both should work well for ES/iPS culture).
10. To make complete media (E8), add to the above E4 medium the following: 0.25ml insulin solution (10mg/ml), 0.5ml FGF2 (100ug/ml), 0.5ml TGFb1 (1.7ug/ml), 0.5ml transferrin (10mg/ml). Mix well. Thaw all additives on cold-rack on ice. Add cold base-media and wait until it is thawed, then add.
11. The complete medium is good for 2 weeks at 4C. DO NOT warm the media before feeding. Warming up the media will destabilize the growth factors in the media.
12. E4 and E6 media is good for at least 3 months at 4C, possibly up to a year.
13. For protocols making aliquots of TFGb1 and transferrin, see next page.

Recombinant Human TGF-beta 1 (R&D 240-B-001MG/CF)

**Most temperature sensitive, work fast

***This is for large number of aliquots. Change volume according to your own scale.

Order 1mg but have them provide it in 2 x 500ug aliquots plus a sample for testing if they will do this. 1000X = 1.745 ug/mL

- For 500ug protein: bring to a total volume of 286.5mLs in buffer
- Use 4mM HCl containing 1mg/mL recombinant human albumin (Sigma A7223)
- Chilled Buffer (make about 300-500mL of buffer)

- For 100mL:

§ 34.4uLs of HCl (11.6M) (in fume hood cabinet) for final concentration of 4mM

§ Add 2 mLs of HSA (50mg/mL) for final 1mg/mL concentration

§ Bring vol up to 100mL with molecular grade water

- For 500mL: 172uL of HCl + 10mL of HSA + 489.83mL of H2O

This can be made at any point and stored at 4C

- Bring Solution to 4 C on ice for 30 mins – do not need to do this if buffer already cold
- Set up tubes in hood – will need 2 hoods (over 500 aliquots)
- To a 250mL Millipore filter, add 100mLs of chilled buffer
- Then add your 500ug bottle of TGFb from R&D Systems (note the total volume – use pipet to measure) – thaw on ice, will take ~30-45min
- For TGFbeta coming in the form of lyophilized powder, dissolve in the chilled buffer at 100ug/ml, then add to appropriate amount of buffer.
- Bring to a total volume of 286.5 mLs
- Filter
- Aliquot 500uL/ tube (get some help because that's over 500 aliquots and you want to work quickly to get them frozen)
- Label

Freeze @ -20C for immediate use or -80C for long term storage

Holo – Transferrin (Sigma T0665-1G) (change scale accordingly if you are making a different amount)

- 1000X = 10.67mg/mL à 426.8mg (+/- 5mg) / 40 mls
- I use chilled PBS to dissolve this
- Static zap your Falcon Tube and weigh paper before transferring the crystals.
- On weigh paper, measure out 426.8mg of Holo-Transferrin
- Add your 40mLs of chilled PBS
- Vortex gently until in solution, avoid foaming.
- Filter with Steriflip
- Aliquot 500uL/ tube
- Label
- Parafilm bottle of holo-transferrin when done
- The solution will be red in color
- If you freeze it at -80C it will be orange in color
- If you freeze it at -20C it will turn clear
- If you freeze it at -80C and move it to -20 C it will slowly turn from orange to clear
- The color is an indicator of the oxidation state, either way it's all right to use