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Making E8 medium for ES/iPS culture

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ABSTRACT

This protocol is about making E8 medium for ES/iPS culture.

ATTACHMENTS

MakingE8fromDMEM-F12.pdf

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MATERIALS TEXT

Preparation of TGFb1:

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

  Albumin human **Sigma**

- **Aldrich Catalog #A7223** Step 1
- 11.6 M HCl
- molecular grade water
- Millipore filters

Preparation of Transferrin:

  holo-Transferrin human **Sigma**

- **Aldrich Catalog #T0665-1G** Step 13
- Millipore Steriflip filter
- PBS

Additional reagents for making E8 medium:

- sodium bicarbonate
- sodium selenite

  L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate **Sigma**

- **Aldrich Catalog #A8960** Step 26
- DMEM/F12.
- Insulin solution (10 mg/ml)
- FGF2 (100 µg/ml)

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

Preparation of Recombinant Human TGF-beta 1 30m

1 Albumin human **Sigma**
Aldrich Catalog #A7223

**Most temperature sensitive, work fast
***This is for large number of aliquots. Change volume according to your own scale.

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

- For 500 µg protein: bring to a total volume of 286.5 mL in buffer
- Use [M]**4 Milimolar (mM) HCl** containing [M]**1 mg/mL recombinant human albumin**
- Chilled Buffer (make about 300-500 mL of buffer)

2

Prepare buffer:

For 100 mL:

1. **34.4 µl HCl (11.6M)** (in fume hood cabinet) for final concentration of [M]**4 Milimolar (mM)**

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2. Add **2 mL HSA (50 mg/mL)** for final **1 mg/mL** concentration
3. Bring vol up to 100 mL with molecular grade water

For 500 mL: **172 µl HCl** + **10 mL HSA** + **489.83 mL H2O**

This can be made at any point and stored at **4 °C** .

3 Bring Solution to **4 °C** **On ice** for **00:30:00** – do not need to do this if buffer already cold. 30m

4 Set up tubes in hood – will need 2 hoods (over 500 aliquots).

5 To a 250 mL Millipore filter, add **100 mL chilled buffer** .

6  45m

Then add your **500 µg bottle of TGFb from R&D Systems** (note the total volume – use pipet to measure) – thaw **On ice** , will take around **00:30:00** - **00:45:00** .

7 For TGFbeta coming in the form of lyophilized powder, dissolve in the chilled buffer at **100 µg/ml** , then add to appropriate amount of buffer.

8 Bring to a total volume of 286.5 mL.

9 Filter.

10 Aliquot **500 µl** /tube (get some help because that's over 500 aliquots and you want to work quickly to get them frozen).

11 Label.

12 Freeze @ **-20 °C** for immediate use or **-80 °C** for long term storage.

Preparation of Holo-Transferrin

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13 holo-Transferrin human **Sigma Aldrich Catalog #T0665-1G**

Change scale accordingly if you are making a different amount.

- 1000X = 10.67 mg/mL à 426.8 mg (+/- 5mg) / 40 ml
- Use chilled PBS to dissolve this

14 Static zap your Falcon Tube and weigh paper before transferring the crystals.

15 On weigh paper, measure out **426.8 mg Holo-Transferrin** .

16 Add your **40 mL chilled PBS** .

17 Vortex gently until in solution, avoid foaming.

18 Filter with Steriflip.

19 Aliquot **500 µl** / tube.

20 Label.

21 Parafilm bottle of holo-transferrin when done.

The solution will be red in color.

- If you freeze it at **-80 °C** it will be orange in color
- If you freeze it at **-20 °C** it will turn clear
- If you freeze it at **-80 °C** 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

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22 Dissolve **1.358 g sodium bicarbonate** in **50 mL water** . Warm the water at **37 °C** to completely dissolve the salt.

23 Dissolve **10 mg sodium selenite** in **1 mL water** . Then dilute this solution 1:100 with water to make **100 µg/ml** solution.

24 

Add **350 µl 100 µg/ml sodium selenite solution** to the **50 mL sodium bicarbonate solution** . Then filter sterilize.

25 Aliquot into 10 ml aliquots. Each aliquot is enough for 500 ml of DMEM/F12.

26 Dissolve **160 mg L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate** (L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate **Sigma Aldrich Catalog #A8960**) in **5 mL water** . Filter sterilize. Then aliquot into 1 ml aliquot. Each aliquot is enough for **500 mL DMEM/F12** .

27 Freeze all aliquots at **-80 °C** .

28 

To make E4 media. Add **10 mL sodium bicarbonate and sodium selenite solution** and **1 mL VitC solution** from above to **500 mL DMEM/F12** .

29 E4 media made this way should have osmolarity around 310 and pH around **7.4** .

30 

To make E6 media, add to the above E4 medium the following: **0.25 mL insulin solution (10 mg/ml)** and **0.5 mL transferrin (10 mg/ml)** . Mix well.

(Note: The original E8 formula contains **20 µg/ml inuslin** . This recipe contains **5 µg/ml insulin** . Both should work well for ES/iPS culture).

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31

To make complete media (E8), add to the above E4 medium the following:
25 mL insulin solution (10 mg/ml) , **0.5 mL FGF2 (100 µg/ml)** , **0.5 mL TGFb1 (1.7 µg/ml)** , **0.5 mL transferrin (10 mg/ml)** . Mix well. Thaw all additives on cold-rack **On ice** . Add cold base-media and wait until it is thawed, then add.

32 The complete medium is good for 2 weeks at **4 °C** . **DO NOT** warm the media before feeding. Warming up the media will destabilize the growth factors in the media.

33 E4 and E6 media is good for at least 3 months at **4 °C** , possibly up to a year.

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