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Making E8 medium for ES/iPS culture

In 1 collection

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ABSTRACT

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

ATTACHMENTS

[MakingE8fromDMEM-F12.pdf](#)

MATERIALS

Preparation of TGFb1:

- Recombinant Human TGF-beta 1 (R&D 240-B-001MG/CF)
- Albumin human Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7223
- 11.6 M HCl
- molecular grade water
- Millipore filters

Preparation of Transferrin:

- holo-Transferrin human Merck MilliporeSigma (Sigma-Aldrich) Catalog #T0665-1G
- Millipore Steriflip filter
- PBS

Additional reagents for making E8 medium:

- sodium bicarbonate
- sodium selenite
- L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate Merck MilliporeSigma (Sigma-Aldrich) Catalog #A8960
- DMEM/F12.
- Insulin solution (10 mg/ml)
- FGF2 (100 µg/ml)

OPEN ACCESS

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We use this protocol and it's working

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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 Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7223

Note

**Most temperature sensitive, work fast

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

Note

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 millimolar (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 millimolar (mM)
2. Add 2 mL HSA (50 mg/mL) for final [M] 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 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 .

45m

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

13 holo-Transferrin human Merck MilliporeSigma (Sigma-Aldrich) Catalog #T0665-1G

Note

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.

Note

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

Making E8 medium for ES/iPS culture

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 μ L 100 μ g/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 Merck MilliporeSigma (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

(Note: The original E8 formula contains $20\text{ }\mu\text{g/ml}$ insulin. This recipe contains $5\text{ }\mu\text{g/ml}$ insulin. Both should work well for ES/iPS culture).

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 ($100\text{ }\mu\text{g/ml}$)

0.5 mL TGFb1 ($1.7\text{ }\mu\text{g/ml}$)

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 $4\text{ }^{\circ}\text{C}$. **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 $4\text{ }^{\circ}\text{C}$, possibly up to a year.