[Protocol 1.07] Preparation of E8 medium

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Media and Reagents:
DMEM/F12 with L-glutamine/HEPES (Corning, 1L, 10-092-CM)
or alternatively
DMEM/F12 HEPES (Thermo Fisher, 500 ml, 11330032)
L-Ascorbic acid 2-phosphate (Sigma, A8960)
Insulin (CS Bio, C9212-1G or Sigma, 91077C-1G)
Transferrin human (Sigma,T3705-1G)
Sodium Selenite (Sigma, S5261-10G)
FGF2 (PeproTech, 100-18B)
TGFβ1 (PeproTech, 100-21C)
Sodium Bicarbonate 7,5% solution (Fisher Scientific, 25080-094)
Distilled Water (Thermo Fisher, 15230162)
PBS w/o Ca^{2+}, Mg^{2+} (Thermo Fisher, 14190144)
HCL 1M (Sigma, H9892-100ml)
NaOH 10M (Sigma, 72068-100ml)

Materials and Equipment:
5/10/25/50 ml serological pipettes
50 ml falcon tubes
5 ml Eppendorf tubes (VWR, 525-0794)
1,5 ml and 2 ml Low protein binding tubes
3 ml one-way drop pipette
250 ml filter bottle with
0,22 µM filter (VWR, 514-0027)

1. Introduction and Purpose

This protocol describes the procedure of preparing homemade E8 Supplement (All-in-one) and home-made E8 medium for hiPSC maintenance culture.

2. Storage conditions
Note*: Upon receipt, store ingredients under appropriate storage conditions.*

Ingredient	Storage	Appearance
L-ascorbic acid 2-phosphate	Room temperature	Powder
Insulin (human recombinant - *E. coli*)	4°C	Powder
Transferrin (human recombinant - rice)	4°C	Powder
Sodium Selenite	Room temperature (Hazardous substance!)	Powder
FGF2 (human recombinant - *E. coli*)	-20°C / long term -80°C	Lyophilisate
TGFβ1 (human recombinant - CHO)	-20°C / long term -80°C	Lyophilisate
DMEM/F12 with L-glutamine/HEPES	4°C	liquid
Sodium Bicarbonate 7,5%	4°C	liquid

3. Preparation of E8 Supplement (All-in-one)
125 ml E8 supplement (All-in-one) is sufficient for preparation of 50 L home-made E8 medium!

Ingredient	125 ml E8 supplement (All-in-one)	Resolve into	Per 1 ml DMEM/F12	Per 1000 ml DMEM/F12
L-ascorbic acid 2-phosphate	3,2 g	Sterile water	64 µg	64 mg
Insulin (human recombinant - *E. coli*)	1 g	Sterile water	20 µg	20 mg
Transferrin (human recombinant - rice)	535 mg	Sterile water	10,7 µg	10,7 mg
Sodium Selenite (700 µg/ml stock solution, -20°C) see appendix	1 ml	Sterile water	14 ng	14 µg
FGF2 (human recombinant - *E. coli*)	5 mg (5x 1 mg)	Cold PBS w/o Ca^{2+}, Mg^{2+}	100 ng	100 µg
TGFβ1 (human recombinant - CHO)	100 ug	Sterile water	2 ng	2 µg
Sodium Bicarbonate (7,5 %)	-	-	7,2 µl	7,2 ml

3.1. L-Ascorbic acid 2-phosphate

Pour 50 ml of distilled water into a clean and sterile 100 ml glass beaker. Gradually introduce 3.2 g of L-ascorbic acid 2-phosphate, and stir the mixture using a stirring bar and plate until it becomes clear.

3.2. Insulin, Transferrin and Sodium Selenite

1. Pour 45 ml of distilled water into a clean and sterile 100 ml glass beaker equipped with a stirring bar placed on a stirring plate.
2. While stirring gently, add 1 g of Insulin to the water. As you do so, the solution will turn white.
3. To dissolve the Insulin completely, begin by adjusting the pH to 3 using a pH-Meter and 1M HCl. Initially, aim for a pH of 4.5 and gradually add approximately 13 drops of 1M HCl until the solution becomes clear.
4. After dissolving the Insulin completely, restore the pH to 7.4. Add around 3 drops of 10M NaOH to achieve this.
(**Note**: Don't be alarmed if the solution turns white again around pH 6; it will quickly clear up afterward.)
5. While continuing to stir, add 535 mg of Transferrin (which has an orange color) and 1 ml of the Sodium Selenite stock solution (at a concentration of 700 µg/ml).
6. Next, employ a 50 ml serological pipette to check if the solution reaches a total volume of 50 ml. If it falls short, supplement it with distilled water until it reaches the 50 ml mark.

3.3. FGF2

Pour 24 ml of cold D-PBS (-/-) into a 50 ml Falcon tube, then thoroughly resuspend the contents of 5 vials, each containing 1 mg of FGF2, in the solution.

3.4. TGFβ1

Mix 1 ml of distilled water with one vial containing 100 µg of TGFβ1.

3.5. Final mixture

Combine all four solutions (**3.1. – 3.4.**) and then sterilize by filtering through a 125 ml filter bottle equipped with a 0.22 µm filter.

3.6. Aliquoting of homemade E8 Supplement

- Aliquot for 1 L medium:
  Make 50 x 2.5 ml aliquots in 5 ml Eppendorf tubes (best: low protein binding) and store at -20 °C.
- Aliquot for 500 ml medium:
  Make 100 x 1.25 ml aliquots in 2 ml Eppendorf tubes (best: low protein binding) and store at -20 °C.

4. Preparation of E8 home-made medium

1. Begin by thawing one aliquot of E8 supplement (All-in-one) (2.5 ml) at room temperature for a few minutes.
2. Once thawed, add the supplement to one bottle of 1L DMEM/F12 with L-glutamine/HEPES media. Additionally, include 7.2 ml of 7.5% Sodium Bicarbonate solution.
Alternatively, if using a smaller volume:

1.Thaw one aliquot of E8 supplement (All-in-one) (1.25 ml) at room temperature for a few minutes.
2. Then, add this supplement to one bottle of 500 mL DMEM/F12 with L-glutamine/HEPES media. Along with it, add 3.6 ml of 7.5% Sodium Bicarbonate solution.

(**Note**: Remember to store the medium in the refrigerator and use it within two weeks for optimal results!)

Appendix

Preparation of Sodium Selenite stock solution (700 µg/ml):

Dilute 70 mg of Sodium Selenite under sterile conditions in 100 ml of distilled water.
Prepare 1 ml aliquots in 1.5 ml Eppendorf tubes (best ultra-low binding) and store at -20°C until usage.

Original recipe / publication:

Chen G, Gulbranson DR, Hou Z, Bolin JM, Ruotti V, Probasco MD, Smuga-Otto K, Howden SE, Diol NR, Propson NE, Wagner R, Lee GO, Antosiewicz-Bourget J, Teng JM, Thomson JA. Chemically defined conditions for human iPSC derivation and culture. *Nat Methods.* 2011 May;8(5):424-9. doi: 10.1038/nmeth.1593. Epub 2011 Apr 10. PubMed PMID: 21478862;