

May 23, 2025

E8 media production

 In 1 collection

DOI

dx.doi.org/10.17504/protocols.io.6qpvr32ppvnmk/v1



Valeria Fernandez Vallone¹, Lyn Healy², Nathalie Lefort³, Katarzyna Ludwik¹, Tamer Onder⁴, Lisa Pavinato^{5,6}, Fatma Visal FVO OKUR⁷, Harald Stachelscheid¹

¹Core Unit pluripotent Stem Cells and Organoids - Berlin Institute of Health @ Charite, Berlin, Germany;

²The Francis Crick Institute;

³Université de Paris, Imagine Institute, iPSC Core Facility, INSERM UMR U1163, F-75015 Paris, France.;

⁴Koc University;

⁵Institute of Oncology Research (IOR), Bellinzona Institutes of Science (BIOS+), Bellinzona, Switzerland;

⁶Faculty of Biomedical Sciences, Università della Svizzera Italiana, Lugano, Switzerland.;

⁷Hacettepe University, Center for Stem Cell Research and Development (PEDISTEM) and Hacettepe University Faculty of Medicine, Department of Pediatrics



Harald Stachelscheid

Core Unit pluripotent Stem Cells and Organoids - Berlin Inst...

OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.6qpvr32ppvnmk/v1

Protocol Citation: Valeria Fernandez Vallone, Lyn Healy, Nathalie Lefort, Katarzyna Ludwik, Tamer Onder, Lisa Pavinato, Fatma Visal FVO OKUR, Harald Stachelscheid 2025. E8 media production. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.6qpvr32ppvnmk/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: November 21, 2023



Last Modified: May 23, 2025

Protocol Integer ID: 91258

Keywords: medium, cell culture, hiPSC, hPSC, E8, home made, defined conditions

Funders Acknowledgements:

COST Action CorEuSTem

Grant ID: CA20140

Abstract

This protocol describes the procedure to prepare E8 Supplement and complete E8 medium for hPSC maintenance culture.

Guidelines

We recommend storage of all the media components in their recommended storage conditions up to one year. Similarly, we recommend that the prepared E8 supplement be stored in -80°C up to one year.



Materials

LABORATORY EQUIPMENT AND CONSUMABLES

Use sterile material

- 5/10/25/50 mL serological pipettes
- 50 mL conical tubes
- 1.5/2/5 mL tubes (low binding protein recommended)
- 10/200/1000µL tips and micropipettes
- 250 mL bottles
- 250 mL bottle with 0.22µm sterile filter
- Analytic scale
- Glass beakers
- Magnetic stirring device and bars

MEDIA AND REAGENTS

⊗ Insulin, human recombinant **Merck MilliporeSigma (Sigma-Aldrich) Catalog #91077C-1G**

Alternatively: Insulin, CSBio, Cat. C9212-1G

⊗ DMEM/F-12, HEPES **Thermo Fisher Scientific Catalog #11330032**

⊗ L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A8960**

⊗ Transferrin human **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T3705**

⊗ Sodium selenite **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S5261**

⊗ Recombinant Human FGF-basic (154 a.a.) **peprotech Catalog #100-18B**

⊗ Recombinant Human TGF-β1 (CHO derived) **peprotech Catalog #100-21C**

⊗ Sodium Bicarbonate 7.5% solution **Thermo Fisher Catalog #25080094**

⊗ Distilled Water **Thermo Fisher Catalog #15230162**

⊗ Gibco™ DPBS no calcium no magnesium **Thermo Fisher Scientific Catalog #14190144**

⊗ Hydrochloric acid solution 1.0 N **Merck MilliporeSigma (Sigma-Aldrich) Catalog #H9892**

⊗ 10M sodium hydroxide solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #72068**



Safety warnings

❗ **Sodium selenite** is considered a hazardous substance. It is toxic and can be harmful if ingested, inhaled, or absorbed through the skin. It is also classified as an environmental hazard, particularly harmful to aquatic life.

Reagent Storage Requirements Upon Delivery

1

A	B	C
Ingredient	Storage	Appearance
L-ascorbic acid 2-phosphate	RT	Powder
Insulin (human recombinant - <i>E. coli</i>)	4°C	Powder
Transferrin (human recombinant - rice)	4°C	Powder
Sodium Selenite	RT	Powder
FGF2 (human recombinant - <i>E. coli</i>)	-20°C / -80°C	Lyophilisate
TGFβ1 (human recombinant - CHO)	-20°C / -80°C	Lyophilisate
DMEM/F12 with L-glutamine/HEPES	4°C	Liquid
Sodium Bicarbonate 7,5%	4°C	Liquid

Table 1. Reagents storage upon delivery


Note

Storage conditions should follow manufacturer instructions. For FGF2 and TGFβ1 long term storage is recommended at -80°C.

Preparation of reagents


1h 28m

2 L-Ascorbic acid 2-phosphate

2.1 Weight  3.2 g of L-ascorbic acid 2-phosphate using analytical scale.

5m



2.2 Add  50 mL of distilled water in a 100 mL clean glass beaker.

2m

2.3 Slowly add prepared L-ascorbic acid 2-phosphate to the water in the glass beaker.

3m




2.4 Stir solution until it is clear.

10m


Note

Do not store this solution. Prepare it fresh.

3 Sodium Selenite stock solution 700 µg/mL

3.1 Dilute  70 mg of Sodium Selenite in  100 mL distilled water to prepare a  700 µg/mL solution


15m

3.2 Prepare  1 mL aliquots in 1.5 mL tubes

10m

3.3 Store at  -20 °C until usage


4 Insulin, Transferrin and Sodium Selenite

4.1 Add  45 mL of distilled water to a 100 mL clean glass beaker containing a stirring bar.

2m

4.2 Place the beaker on a stirring plate and apply low speed.

1m

4.3 Add  1 g of Insulin, the solution becomes white.

3m

4.4 To dissolve Insulin adjust to  3 adding drop-wise HCl  1 Molarity (M) .

5m







4.5 When solution gets clear, adjust  7.4 adding drop-wise NaOH  10 Molarity (M) .

2m

4.6 Keep stirring the solution the whole time.

Note


Expect a starting  4.5 and use about 13 drops HCL. Adjusting back  7.4 will need about 3 drops NaOH. At ~  6 the solutions gets white again but clears up immediately after.

4.7 While stirring Insulin solution, add  535 mg of Transferrin (expect the powder to be orange)

5m

4.8 Add  1 mL of Sodium Selenite stock solution prepared in step 3

2m

4.9 Use a 50 mL serological pipette to measure the solution volume and adjust to  50 mL by adding distilled water.

5m


Note

Do not store Insulin, Transferrin and Sodium Selenite solution long term. Prepare fresh.

5 **Human recombinant FGF2 Stock** 200 µg/mL

5.1 Add  24 mL of cold DPBS (-Mg₂/-Ca₂) to a 50 mL tube

1m

5.2 Re-suspend the content of 5 vials of  1 mg vials of hrFGF2

5m

5.3 Transfer all to 50 mL tube rinsing original vials

5m



5.4 Mix the solution by pipetting up and down using 10 mL pipette

3m

**Note**

Keep recombinant proteins always on ice upon preparation.
If needed, stock solution can be aliquoted and stored at -80°C.

6 Human recombinant TGFβ1 Stock [M] 100 µg/mL

6.1 Add  1 mL of cold distilled water to  100 µg vial of hrTGFβ1

2m

6.2 Mix the solution by pipetting up and down using microtiter 1000µl pipette

2m

Note

Keep recombinant proteins always on ice upon preparation.
If needed, stock solution can be aliquoted and stored at -80°C.


E8 Supplement

25m

7 Assembly of E8 Supplement

7.1 Mix all solutions prepared in steps 2, 4, 5 and 6 in a 250 mL clean bottle

5m

7.2 Filter sterile using filter bottle with  0.22 µm filter

5m

7.3 Prepare aliquots according to the table below:

15m

A	B	C
Aliquot size	2.5 mL	1.25 mL
Final media volume	1 L	0.5 L

Table 2. E8 supplement aliquot size per final volume of E8 media to be prepared.



Note

Store E8 supplements aliquots at $-80\text{ }^{\circ}\text{C}$ until usage.
If available use low binding protein tubes for E8 supplement aliquoting and storage.

Supplemented E8 media

16m

8 Preparation of supplemented E8 media (0.5 L)

8.1 Thaw one aliquot 1.25 mL of E8 supplement prepared in step 7.

10m

8.2 Add 1.25 mL E8 supplement to 0.5 L of DMEM/F12, HEPES.

2m

8.3 Add 3.6 mL of 7.5 \% volume sodium Bicarbonate solution.

2m

8.4 Mix thoroughly all ingredients for a homogeneous composition.

2m

Note

Store supplemented E8 media at $4\text{ }^{\circ}\text{C}$ for up to 2 weeks.

E8 Batch quality control

9 Perform hPSC maintenance for at least 3 passages using 2 hPSC lines for which culture behaviour is well characterized in-house. Compare side by side validated E8 media batch with newly prepared one.

Note

Refer to protocol: **Maintenance of hPSC**.

10 Monitor the culture thorough the process and register hPSC morphology taking images for documentation of batch validation. Refer to protocol **Reference pictures of hPSC**



cultured in defined conditions for typical morphologies in different media/matrix conditions.

Protocol references

Chen G, Gulbranson D, Hou Z, et al. Chemically defined conditions for human iPSC derivation and culture. *Nat Methods*. 2011;8(5):424–429. <https://doi.org/10.1038/nmeth.1593>