

SOP iPSC PT02-3v1	Title: Preparation of Culture Media
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Date: 24.4.15	Date: 24.04.15	Date: 29.04.15

OBJECTIVE

This SOP describes the preparation of several media used in human and murine pluripotent stem cell culture.

PREPARATION OF HES CULTURE MEDIUM

Reagents:

- KO DMEM (Invitrogen #10829-018) (4°C)
- KO SR (Invitrogen #10828-028) (-20°C)
- MEM NEEA 100X (Cambrex #13-114) (4°C)
- 2-Mercaptoethanol 50mM (Invitrogen #31350-010) (4°C)
- Penicilin(10.000U/ml)/Streptomycin(10.000ug/ml)(Invitrogen#15140-122) (-20°C)
- GlutaMAX 200mM (Invitrogen #35050-038) (4°C)
- *bFGF 1000µg (Millipore #GF003AF-MG) (-20°C)

**preparation of bFGF:*

- bFGF 1000µg (-20°C)
- Human Serum Albumin solution (HSA) 100mg/ml (10% en P/V)(4°C) (Vitrolife# 10064)
- Phosphate buffered saline (PBS) without magnesium and calcium (Cultek, SLU, Catalog #17-516F)
- SARSTEDT tubes (1.5ml) (Sarstedt # 72.692.005)

1. Centrifuge the lyophilized bFGF for 1-2 minutes at 10.000 rpm, to concentrate bFGF on the the bottom of the tube.
2. Prepare 0.2% HSA in PBS in a sterile tube (1:50 dilution).
3. Add 10ml of the HSA 0.2% solution to the bFGF tube to obtain a final concentration of 100 µg/ml.
4. Prepare aliquots in SARSTEDT tubes indicating name, concentration and preparation date.
5. Freeze at -20°C

To filter the medium:

- MILLIPORE Express PLUS filter unit 500ml (0.22 µm) (Millipore #SCGPU05RE).

For 500ml:

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|-------------------|--------|
| -KO DMEM | 387 ml |
| -20% Knockout SR: | 100 ml |

-1x NEEA:	5 ml
-50 U/ml-50µg/ml Penicilin/Streptomycin (P/S)	2.5ml
-1X Glutamax	5 ml
-50 µM 2-Mercaptoethanol	500 µl
-10 ng/ml bFGF	50 µl

1. Mix all the components in the filter unit MILLIPORE Express PLUS and filter by vacuum aspiration.
2. Identify by labeling bottle with medium name and date of preparation.
3. Store at 4°C.

IMPORTANT: Because HES medium contains many thermo labile ingredients, calculate the exact volume needed, and warm it in a sterile tube at 37°C.

PREPARATION OF HFF-1 CULTURE MEDIUM

Reagents:

- IMDM (Gibco/Invitrogen # 21980-032) (4°C)
- Fetal Bovine Serum (FBS) (Invitrogen # 10270-106) (-20°C)
- Penicilin(10.000U/ml)/Streptomycin (10.000ug/ml)Invitrogen #15140-122) (-20°C)

To filter the medium:

-MILLIPORE Express PLUS filter unit 500ml (0.22 µm) (Millipore #SCGPU05RE).

For 500 ml:

-IMDM	447.5 ml
-10% FBS:	50 ml
-50 U/ml-50µg/ml Penicilin/Streptomycin (P/S):	2.5 ml

1. Mix all the components in the filter unit MILLIPORE Express PLUS and filter by vacuum aspiration.
2. Identify by labeling bottle with medium name and date of preparation.
3. Store at 4°C.

PREPARATION OF MEFs CULTURE MEDIUM

Reagents:

- DMEM high glucose (Invitrogen # 21969-035) (4°C)
- Fetal Bovine Serum (FBS) (Invitrogen # 10270-106) (-20°C)
- Glutamax (Invitrogen #35050-038) (4°C)
- Penicillin/streptomycin (Invitrogen #15140-122) (-20°C)

To filter the medium:

-MILLIPORE Express PLUS filter unit 500ml (0.22 µm) (Millipore #SCGPU05RE).

For 500 ml:

-DMEM high glucose	440 ml
-FBS 10%	50 ml
-Glutamax 1X	5 ml
-Penicillin/Streptomycin 1X	5 ml

1. Mix all the components in the filter unit MILLIPORE Express PLUS and filter by vacuum aspiration.
2. Identify by reporting medium name and date of preparation.
3. Store at 4°C.

G4 MEDIA (for culturing mouse embryonic stem cells):

- DMEM (Gibco # 21969-035) (4°C).
- Fetal Bovine Serum (FBS) (Invitrogen # 10270-106) (-20°C).
- MEM NEAA 100X(Gibco) (4°C).
- Penicillin (10.000U/ml)/Streptomycin (10.000ug/ml) (100X)(Gibco #15140-122) (-20°C)
- GlutaMAX 200mM (Gibco #35050-038) (-20°C).
- Sodium Pyruvate (Gibco #11360) (4°C).
- 2-Mercaptoethanol 50mM (Gibco #31350-010) (4°C).
- LIF 1000U/ml (Chemicon #ESG1107) (4°C).

For 250ml:

-DMEM	205,5ml
-15% FBS	37ml
-1x MEM NEAA	2,5ml
-1x Sodium Pyruvate	2,5ml
-1x penicillin/Streptomycin (100U/ml, 100µg/ml)	1,25ml
-1x GlutaMAX	2,5ml
-50µM 2-Mercaptoethanol	500µl
-LIF 1000U/ml	25µl

PREPARATION OF DERIVATION MEDIUM

Reagents

- KO DMEM (Invitrogen #10829-018). (4°C)
- KO SR (Invitrogen #10828-028). (-20°C)
- MEM NEEA 100X (Cambrex #13-114). (4°C)
- 2-Mercaptoethanol 50mM (Invitrogen #31350-010). (4°C)
- Penicilin(10.000U/ml)/Streptomycin(10.000ug/ml)(Invitrogen #15140-122)(-20°C)
- GlutaMAX 200mM (Invitrogen #35050-038) (4°C)
- FBS ES Cell Certified (HyClone/Cultek #SH30070.02E)
- *bFGF 1000µg (Millipore #GF003AF-MG) (-20°C) (see previous preparation of bFGF detailed above)

Procedure:

Prepare derivation medium as follows:

- 50% of HES medium conditioned during 24h in hESC and supplemented with 2.5% ES-cell tested FBS (Hyclone) (filtered).
- 50% HES medium supplemented with 2.5% Es-cell tested FBS (Hyclone). Add the FGF (10 ng/ml) and the β -mercaptoetanol (50 μ M) daily.

Title:

PREPARATION OF CULTURE MEDIA HES

(Annex Table Volume)

500ml:

-KO DMEM	387 ml
-20% Knockout SR:	100 ml
-1x NEEA:	5 ml
-50 U/ml-50µg/ml Penicilin/Streptomycin (P/S):	2.5 ml
-1X Glutamax:	5 ml
-50 µM 2-Mercaptoethanol	500 µl
-10 ng/ml bFGF	50 µl

250ml:

-KO DMEM	193.5 ml
-20% Knockout SR:	50 ml
-1x NEEA:	2.5 ml
-50 U/ml-50µg/ml Penicilin/Streptomycin (P/S):	1.25 ml
-1X Glutamax:	2.5 ml
-50 µM 2-Mercaptoethanol	250 µl
-10 ng/ml bFGF	25 µl

125ml:

-KO DMEM	96.75 ml
-20% Knockout SR:	25 ml
-1x NEEA:	1.25 ml
-50 U/ml-50µg/ml Penicilin/Streptomycin (P/S):	625 µl
-1X Glutamax:	1.25ml
-50 µM 2-Mercaptoethanol	125 µl
-10 ng/ml bFGF	12.5 µl

PROTECTION MESSURES FOR USING THIS TECHNIQUE

- Avoid wounds and scratches in handling of parts and accessories of instruments that can be sharp and in the access to difficult areas.

- Use of biosafety hoods in combination with additional personal protective equipment (Biological Safety Cabinets Class II will be used).

- Washing hands after handling biological material and before leaving the laboratory.

USE CHEMICAL PRODUCTS

- Use lab coat
- Work in a gas extractor hoods
- Use safety glasses with side protection (EN 166)
- Use nitrile chemical protective gloves (EN 374)
- Remove the gloves without touching the surface of the glove to avoid skin contact with the product
- Throw the gloves in the correct container
- Wash and dry your hands immediately after using the substance