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

preparation of bFGF:

-bFGF 1000µg (-20ºC)
-Human Serum Albumin solution (HSA) 100mg/ml (10% en P/V)(4ºC) (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 -20ºC

To filter the medium:

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

For 500ml:

-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 4ºC.

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

PREPARATION OF HFF-1 CULTURE MEDIUM

Reagents:

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

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 4ºC.

PREPARATION OF MEFs CULTURE MEDIUM

Reagents:

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

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 4ºC.

G4 MEDIA (for culturing mouse embryonic stem cells):

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

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). (4ºC)
-KO SR (Invitrogen #10828-028). (-20ºC)
-MEM NEEA 100X (Cambrex #13-114). (4ºC)
-2-Mercaptoethanol 50mM (Invitrogen #31350-010). (4ºC)
-Penicilin(10.000U/ml)/Streptomycin(10.000ug/ml)(Invitrogen #15140-122)(-20ºC)
-GlutaMAX 200mM (Invitrogen #35050-038) (4ºC)
-FBS ES Cell Certified (HyClone/Cultek #SH30070.02E)
-*bFGF 1000µg (Millipore #GF003AF-MG) (-20ºC**)** (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 µM) daily.

Title:

PREPARATION OF CULTURE MEDIA HES

(Annex Table Volume)

500ml:

-KO DMEM	387 ml
-20% Knocout SR:	100 ml
-1x NEEA:	5 ml
-50 U/ml-50µg/ml Penicilin/Streptomicin (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% Knocout SR:	50 ml
-1x NEEA:	2.5 ml
-50 U/ml-50µg/ml Penicilin/Streptomicin (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% Knocout SR:	25 ml
-1x NEEA:	1.25 ml
-50 U/ml-50µg/ml Penicilin/Streptomicin (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).

- Work benches shall be decontaminated at least once a day and whenever there is a spill.

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

PROTECTION EYES / FACE

- Use of biosafety hoods. If it is not possible, safety goggles should be used in those cases where, by the nature of the procedure performed, splashes affecting the mucous membranes of eyes are expected.

- Face shields should be used in situations of risk where eye protection should be extended to the face.

- The use of surgical masks could be considered sufficient against biological risks coming from splashes. However, these masks are not considered personal protective equipment for the respiratory system.

- Use cryogenic gloves

SKIN PROTECTION

- The continuous use of gloves is mandatory in all operations.

- Hands and arms are normally the parts of the body that more frequently come into contact with sharp objects and splashing. Gloves and sleeves garments are ideal for protecting hands and arms.

- Gloves to protect against biological agents must be waterproof, flexible and with great sensitiveness to enable use in all types of work. When it is required, they should be sterile.

BODY PROTECTION

- The use lab coat is mandatory

RESPIRATORY PROTECTION

-Use of biosafety hoods. If it is not possible use a respiratory mask type P3.

USE CHEMICAL PRODUCTS

ALWAYS CONSULT THE SAFETY DATA SHEETS BEFORE YOU BEGIN WORKING WITH A CHEMICAL PRODUCT

- Use lab coat
- Work in a gas extractor hoods. If you cannot do so, use a respiratory mask
- 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

WHAT TO DO IN CASE OF EMERGENCY: BIOLOGICAL AGENT (leak, spillage, etc.)

In case of a leak, spillage and accident, such as inoculation, cut and pricks to the skin, inform immediately the person in charge of Emergencies, your direct head (Head of Department, Platform or Laboratory), and the person in charge for the Safety at the workplace.

FIRST AID INSTRUCTIONS

After inoculations, cuts or pricks to the skin: A small hemorrhage has to be provoked and the wound has to be washed with water and neutral soap, do not rub, and add some iodized Povidona.

After sprinklings on the skin: Wash the affected area with abundant cold water and neutral soap, without rubbing, for 10 minutes.

After sprinklings in the eyes: Wash the eyes with water in the special basin for the eyes and keep the eyelids open, for 20 minutes.

In all cases and after the first cure, the biological agent involved in the accident and the origin of the leak has to be identified, inform the person in charge for the Safety at the workplace and go to see the doctor of the insurance company for work-related accidents (Mutua).

Biological spillage

Disinfect the area contaminated with a 10 % dilution of lye.

If it is necessary, disinfect the area with antifog fluid.

If the paper forms of the laboratory or other manuscript or printed paper are contaminated, the information shall be copied in another document and the original has to be thrown in the container for contaminated waste.

Emission of potentially infectious aerosols (out of a camera of biological safety)

Everyone should evacuate the affected area immediately; those exposed to the emission should be sent immediately to receive medical attention. Nobody will be able to enter the area during a specified time, so that the aerosols could go out and the heaviest particles settle. If the laboratory is not fitted with a central air evacuation system, the access will be delayed.

Signs will be placed indicating that entry is forbidden. After the appropriate time, the decontamination has to be done under the supervision of the person in charge of the Laboratory. For this it will be necessary to use protective clothes and suitable breathing equipment.

WHAT TO DO IN CASE OF EMERGENCY: SPILLAGE OR EMISSION OF CHEMICAL SUBSTANCES

Spillage of a Chemical Substance

- Notify the situation to the person in charge of Emergencies, your direct head (Head of Department or Platform), or the Waste Supervisor. Alert the employees who work nearby.

- Protect yourself against the possible risks that could be caused by the chemical substance. Do not take action if you do not have facial, respiratory and skin protection (gloves and suit).

- In the case of small quantities, control the spillage the nearest to the possible origin. Use the adequate absorbent method for the product spilled. (KIT SEKUROKA)

- Ensure that the product doesn’t enter the drainage system or closed rooms.

- Remove the product, following the instructions given in the Safety File.

- If the spillage is great, notify the person in charge of Emergencies, your direct head (Head of Department or Platform), the Waste Supervisor or the Safety Control Centre of the PRBB and get away from there.

Emission of substances

- If, when entering a zone where chemical products are stored you detect an intense smell or your eyes, nasal mucous or respiratory tract start itching while you are in the room:

- Leave the zone.

- Close the entrance.

- Notify the incident to the person in charge of Emergencies, your direct head (Head of Department or Platform), the Waste Supervisor or the Center of Control of the PRBB, indicating the characteristics of the accident and the location of the same.

FIRST AID

In case of inhalation: quickly breathe in fresh air.

In case of contact with skin: remove contaminated clothing and wash the affected area with plenty of water, without rubbing, for 15 minutes.

In case of contact with eyes: wash eyes with eye bath using plenty of water with the eyelids open for 15 minutes.

In all cases, once immediate action has been taken, inform the work hazards prevention service and visit Mútua d'Accidents de Treball i Malalties Professionals (Work Accidents and Occupational Diseases Mutual Benefit Society) doctor.