

REAGENTS AND MEDIUM PREPARATION

STEM PRO hESC MEDIUM

DMEM/F12 (1x) with GlutaMAX, Life Technologies # 31331-028/10565-018
STEM PRO hESC Supplement (1x), Life technologies # A10006-01
Bovine Serum Albumin (25%), Life technologies #A10008-01
2-mercaptoethanol (50mM), Life technologies #31350-010
bFGF(100 µg/ml), Sigma #F0291 or Life technologies #PHG0026 (50 µg),
#PHG0021 (100 µg)

Medium preparation (final concentration in medium) for 50ml:

50 ml

45.4 ml DMEM/F12 with GlutaMAX (1x)
1 ml STEM PRO hESC Supplement (1x)
100 µl 2-mercaptoethanol (0.1 mM)
3.6 ml Bovine Serum Albumin (1.8 %)
4 µl bFGF (8 ng/ml)

Add all components into a filter bottle/syringe (pore size 0,22 µm) and sterile filtrate. Store at +4 °C in the dark for up to 7 days.

HES MEDIUM

DMEM/F12 (1x) with GlutaMAX, Life Technologies # 31331-028
KnockOut Serum Replacement (100%), Life technologies #10828-028
2-mercaptoethanol (50mM), Life technologies #31350-010
Non-Essential Amino Acids (NEAA) (100x), Life technologies #11140-035
bFGF(100 µg/ml), Sigma #F0291 or Life technologies #PHG0026 (50 µg),
#PHG0021 (100 µg)

Optional

ITS Liquid Medium Supplement (100x), Sigma #13146
Penicillin-Streptomycin (100x), Life Technologies #15140-122

Medium preparation (final concentration in medium) for 50ml:

50 ml

40,5 ml DMEM/F12 with GlutaMAX (1x)
10 ml KO SR (20 %)
92 µl 2-mercaptoethanol (0,0915mM)
0,5 ml NEAA (1x)
3 µl bFGF (6 ng/ml)
0,5 ml ITS (1x) – **optional**
0,5 ml Penicillin-Streptomycin (1x) – **optional**

Add all components into a filter bottle/syringe (pore size 0,22 µm) and sterile filtre. Store at +4 °C.

MEF MEDIUM

DMEM (1x), Sigma #6546
FBS (100%). Life Technologies # 10106-169
GlutaMAX Supplement (100x), Life technologies #35050-038

Optional

Penicillin-Streptomycin (100x), Life Technologies #15140-122

Medium preparation (final concentration in medium) for 50ml:

50 ml

45 ml DMEM (1x)
5 ml FBS (10 %)
0,5 ml GlutaMAX (1x)

0,5 ml Penicillin-Streptomycin (1x) – **optional**

Add all components into a filter bottle/syringe (pore size 0,22 µm) and sterile filtre. Store at +4 °C.

FREEZING MEDIUM FOR HFFs AND MEFs

Medium components:

Stock conc.

	Supplier	Cat. Number
MEF medium		
FBS	Life Technologies	# 10106-169
Dimethyl Sulphoxide (DMSO)	Sigma	# D2650

Medium preparation, 10ml:

10ml	Volume
MEF medium	6ml
FBS	2ml
DMSO	2ml

Add MEF medium and FBS to a 10ml Falcon tube. Filter sterilize at 0.22µm. Add DMSO. Store at +4 °C.

FREEZING MEDIUM I and II FOR HPSC

I Freezing medium components:

Stock conc.

	Supplier	Cat. number
DMEM (1x)	Sigma	# 6546
FBS	Life Technologies	# 10106-169

II Freezing medium components:

Stock conc.

	Supplier	Cat. Number
DMEM (1x)	Sigma	# 6546
FBS	Life Technologies	# 10106-169
Dimethyl Sulphoxide (DMSO)	Sigma	# D2650

Storage conditions:

DMEM => +4°C

FBS => +4°C/-20°C

DMSO => RT

Preparation:

I Freezing Medium preparation, 10ml:

10ml	Volume
DMEM	8ml
FBS	2ml

Add DMEM and FBS to a 10ml Falcon tube. Filter sterilize at 0.22 μ m.

II Freezing Medium preparation, 10ml:

10ml	Volume
DMEM	6ml
FBS	2ml
DMSO	2ml

Add DMEM and FBS to a 10ml Falcon tube. Filter sterilize at 0.22 μ m. Add DMSO. Store at +4 °C.

ROCK INHIBITOR, Y-27632

Components:

Rock-inhibitor, Wako Chemicals Cmbit # 257-00511; CAS 146986-50-7
 1x PBS

Preparation:

1 mg/ml Rock-inhibitor => (stock concentration)
 3 ml 1x PBS

Add 3 ml 1x PBS to Rock-inhibitor vial. Dissolve and aliquote about 100 μ l/ eppendorf tube. Store at -20 °C.

PINACIDIL 1000X

Components:

Pinacidil, Sigma # P154 100 mg (mw. 263.34)
 Dimetyl Sulphoxide (DMSO), Sigma # D2650

Preparation:

Dissolve 100 mg Pinacidil to 3.8 ml DMSO and aliquote 200 μ l/ Eppendorf tube. Store at -20 °C.

COLLAGENASE IV (~ 2000U/ml)

Components:

Collagenase IV, Life Technologies # 17104-019
DMEM/F12 (1x) with GlutaMAX, Life Technologies # 31331-028

Preparation:

1 g (~ 200 U/mg) Collagenase IV
100 ml DMEM/F12
→ ~ 2000U/ml (stock concentration) = 10x (~ 10 mg/ml)

Dissolve ~ 20 000 units of Collagenase IV in 100 ml DMEM/F12. Sterile filter (0.22 µM) and aliquot 1 ml per tube. Store -20 °C

Usage:

Use 1x Collagenase IV (1 mg/ml) in passaging ES/hPS cells. Make 1x Collagenase by diluting 10x Collagenase IV with DMEM/F12. 1x Collagenase IV can be stored at 4 °C for two weeks. Store in dark.

MATRIGEL™ BASEMENT MEMBRANE MATRIX

Components:

Matrigel™ basement membrane matrix growth factor reduced, phenol red free BD,
356231 10 ml

Preparation:

To prepare Matrigel™ aliquots, slowly thaw on ice at +4 °C overnight to avoid the formation of a gel.
Aliquot 200 µl into each tube, keep the bottle and tubes on ice. Store at -20 °C (stable for a minimum of 3 month).

Usage:

Use for preparation of Matrigel™ coated cell culture dishes and plates

1. **Slowly** thaw Matrigel™ aliquot(s) **on ice** at +4 °C.
2. Dilute the Matrigel™ into 40 ml (final dilution of 1:200) of **cold** serum-free DMEM/F12 media.
3. Coat cell culture dishes/plates with diluted Matrigel™ solution as indicated below.

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BIOMEDICUM
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4. Incubate dishes/plates 2 h at RT or +4 °C overnight.
5. Remove Matrigel™ solution immediately before use. Rinse dishes/plates gently once with serum-free DMEM/F12 media before adding cell culture media.
6. The dishes and plates with Matrigel™ solution can be stored at +4 °C for four weeks. Seal dishes and plates with parafilm to prevent dehydration.

Amount of Matrigel™ used per cell culture vessel:

6 well plate	1.5 ml/well
12 well plate	1 ml/well
24 well plate	0.5 ml/well
48 well plate	0.2 ml/well
96 well plate	0.04 ml/well
3.5 cm dish	1.5 ml/dish
6 cm dish	3 ml/dish
10 cm dish	8 ml/dish

MITOMYCIN C

Components:

Mitomycin C, Roche # 10107409001 2 mg

Preparation:

2 mg Mitomycin C
2 000 µl 1x PBS (w/o Ca²⁺/Mg²⁺)
→ 1 000 µg/ml (stock concentration)

1. Add 2 000 µl 1x PBS (w/o Ca²⁺/Mg²⁺) into the Mitomycin C bottle using a syringe **(DO NOT remove the rubber covering!)**. Do not remove the syringe at this stage!
2. Remove the Mitomycin C solution from the bottle with another syringe.
3. Filter the solution using a 0.22 µm syringe filter.
4. Aliquote the solution into appropriate volumes (e.g. 200 µl/tube).
5. Store at -20 °C.

Usage:

Use for inactivating MEFs and HFFs. Add Mitomycin C solution (1 000 µg/ml) in MEF media such that the final concentration is 2 µg/ml (20 µl Mitomycin C per 10 ml of MEF media).

1x TRYPSIN-EDTA

Components:

10x Trypsin-EDTA , Sigma # T 4174
1x PBS (w/o Ca²⁺/Mg²⁺)

Preparation:

Aliquot 10x Trypsin-EDTA 5ml per 15 ml tube and store at -20 °C.

Usage:

Make 1x Trypsin-EDTA by diluting 10x Trypsin-EDTA with 1xPBS (w/o Ca²⁺/Mg²⁺)

1xPBS (w/o Ca²⁺/Mg²⁺)

Components:

10x PBS (w/o Ca²⁺/Mg²⁺), Life Technologies # 14200-067
Milli-Q H₂O

Preparation:

100 ml 10x PBS (w/o Ca²⁺/Mg²⁺)
900 ml milli-Q H₂O

Aliquot 100 ml bottles and autoclave before use. Store at RT.

0.1 % GELATIN

Components:

Gelatin, Sigma # G1890
Milli-Q H₂O

Preparation:

0.5 g Gelatin
500 ml milli-Q H₂O

Prepare a 0.1 % solution of gelatin in mQ-water. Ensure that the gelatin is thoroughly dissolved by heating the solution. Autoclave before use. Store at RT.

Usage:

0.1 % gelatin is used to coat cell culture dishes before plating feeder cells on them. Cover the whole surface of the culture plate with 0.1 % gelatin (use 1 ml for 3.5 cm dish and 3 ml for 6 cm dish). Aspirate gelatin from culture plates before plating feeder cells on them.

Preparing KNOCKOUT™ SR aliquots

Components:

KNOCKOUT™ SR, Life Technologies # 10828-028

Preparation:

500 ml KNOCKOUT™ SR (frozen)
+37 °C

Thaw KNOCKOUT™ SR in a +37 °C water bath and swirl frequently. Remove promptly once thawed. Thawed KNOCKOUT™ SR can be stored at +4 °C for one week. If the entire bottle will not be used within one week of thaw date, aliquots should be made and store at -20 °C. Try to avoid repeated freeze/thaw cycles.

IMPORTANT! Do not heat-inactivate KNOCKOUT™ SR. Store in the dark

Preparing FETAL BOVINE SERUM aliquots

Components:

Fetal bovine serum (FBS), Life Technologies # 10270-106

Preparation:

500 ml Fetal bovine serum (frozen)

Thaw Fetal bovine serum in a +4 °C overnight. Aliquot 50 ml and store at -20 °C. Try to avoid repeated freeze/thaw cycles.