

## APPENDIX

### A. Knockout DMEM/F12 and GlutaMAX™ can be substituted with the following alternatives:

i. DMEM/F-12 containing GlutaMAX™-I (Cat. no. 10565-018)

To prepare 100 mL of **complete KnockOut™ SR Feeder-Free (KSR-FF) medium** using DMEM/F-12 containing GlutaMAX™-I (Cat. no. 10565-018), aseptically combine the components listed in the table below.

Component	Stock Concentration	Final Concentration	Volume
DMEM/F12 containing GlutaMAX™-I (Cat. no. 10565-018)	–	1X	77.8 mL
KnockOut™ SR (Cat. no. 10828-028)	–	20%	20 mL
KnockOut™ SR –GFC (Cat. no. A10580-01)	50X	1X	2 mL
bFGF (Cat. no. PHG0024).	10 µg/mL	20 ng/mL	200 µL

ii. Knockout™ DMEM (Cat. No. 10829-018) and GlutaMAX™-I (Cat. No. 35050-061)

To prepare 100 mL of **complete KnockOut™ SR Feeder-Free (KSR-FF) medium** using Knockout DMEM (Cat. No. 10829-018) aseptically combine the components listed in the table below.

Component	Stock Concentration	Final Concentration	Volume
Knockout DMEM (Cat. No. 10829-018)	–	1X	76.8 mL
GlutaMAX™ -I (Cat. No. 35050-061)	200 mM	2 mM	1 mL
KnockOut™ SR (Cat. no. 10828-028)	–	20%	20 mL
KnockOut™ SR –GFC (Cat. no. A10580-01)	50X	1X	2 mL
bFGF (Cat. no. PHG0024).	10 µg/mL	20 ng/mL	200 µL

iii. MEF – Conditioned Medium (MEF-CM)

To prepare 100 mL of **MEF-CM**, aseptically combine the components listed in the table below. This assumes you have **prepared your own CM** using Knockout DMEM (Cat. No. 10829-018), 20% KnockOut™ SR (Cat. no. 10828-028) and 1X NEAA (11140-050).

Component	Stock Concentration	Final Concentration	Volume
Conditioned Medium	–	1X	98.8mL
GlutaMAX™ -I (Cat. No. 35050-061)	200 mM	2 mM	1 mL
bFGF (Cat. no. PHG0024).	10 µg/mL	20 ng/mL	200 µL

iv. KnockOut™ SR (Cat. no. 10828-028) with feeders

To prepare 100 mL of **complete medium for use with feeders**, aseptically combine the components listed in the table below.

Component	Stock Concentration	Final Concentration	Volume
Knockout DMEM (Cat. No. 10829-018)	–	1X	77.8 mL
GlutaMAX™ -I (Cat. No. 35050-061)	200 mM	2 mM	1 mL
KnockOut™ SR (Cat. no. 10828-028)	–	20%	20 mL
NEAA	10mM	0.1mM	1mL
bFGF (Cat. no. PHG0024).	10 µg/mL	20 ng/mL	200 µL

#### B. Alternative bFGF pack sizes

Product Name	Cat. no.	Product Size
FGF-basic (AA 10-155) Recombinant Human	PHG0021	100 µg
FGF-basic (AA 10-155) Recombinant Human	PHG0023	1 mg
FGF-basic (AA 10-155) Recombinant Human	PHG0024	10 µg
FGF-basic (AA 10-155) Recombinant Human	PHG0026	50 µg
FGF-basic (AA 10-155) Recombinant Human (Liquid Form)	PHG0021L	100 µg

#### C. Dissociation Enzymes/ Tools for Harvesting hESC/iPSC

Dissociation Enzyme /Tools	Application	Suggested concentration
StemPro® EZPassage™ tool (Cat. no. 23181-010)	Manual passaging	Sterile, disposable tool
StemPro® Accutase® (Cat no. A11105-01)	Monolayer of cells post passage, Dissociation into single cells	1X ready to use (1-2 minutes incubation at 37 C)
Dispase (Cat no. 17105-041)	Colony-like morphology post passage	2mg/ml for 2-3 minutes incubation at 37 C
TrypLE™ Express (Cat no.12604-021)	Dissociation to single cells	1X ready to use

#### D. Geltrex™ can be substituted with CELLstart™, a fully-defined, xeno-free cGMP substrate for attachment and expansion of iPS and ES cells.

Preparing CELLstart™-coated Culture Dishes

1. Dilute CELLstart™ (1 mL) 1:50 in D-PBS containing calcium and magnesium. Pipette the solution gently to mix. **Do not vortex.**
2. Cover the whole surface of each culture dish with the CELLstart™ solution (1 mL for a 35-mm dish, 1.5 mL for a 60-mm dish).

3. Seal each dish with Parafilm to prevent drying, and incubate the dishes for 1–2 hours at 37°C.

4. Transfer each dish to a laminar flow hood and allow it to equilibrate to room temperature (about 1 hour) before use.

**Note:** You may store CELLstart™-coated culture dishes at 4°C for next-day use. Carefully wrap the dishes with Parafilm to prevent from drying.

5. Immediately before use, aspirate all CELLstart™ solution from the culture dishes. It is not necessary to rinse the dishes after removing CELLstart™.

#### E. Alternative dilutions for preparing Geltrex™-coated Culture Dishes

Most customers have seen that a dilution of 1:100 is appropriate for their hESC and hiPSC lines. Some lines may require a different dilution for optimal growth. Try anywhere from 1:30 to 1:200.

<b>Dilution</b>	<b>Geltrex Volume</b>	<b>Basal Medium Volume</b>
1:30	1 mL	29 mL
1:50	1 mL	49 mL
1:150	1 mL	149 mL
1:200	1 mL	199 mL