APPENDIX

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

i. DMEM/F-12 containing GlutaMAX-I (Cat. no. 10565018)

To prepare 100 mL of **complete KnockOut SR Feeder-Free (KSR-FF) medium** using DMEM/F-12 containing GlutaMAX-I (Cat. no. 10565018), 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. 10829018) and GlutaMAX-I (Cat. No. 35050061)

To prepare 100 mL of **complete KnockOut SR Feeder-Free (KSR-FF) medium** using Knockout DMEM (Cat. No. 10829018) 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

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 temperate (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.

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

F. **Adaptation protocols for transitioning to Knockout SR FeederFree (KSR-FF)**

Sequential adaptation - Better results may be obtained by gradually adapting iPS cell lines to KSR-FF

1. Coat plates with Geltrex hESC-Qualified Reduced Growth Factor Basement Matrix as described above
2. Passage 1: 75% MEF-conditioned medium + 25% KSR-FF
3. Passage 2: 50% MEF-conditioned medium + 50% KSR-FF
4. Passage 3: 25% MEF-conditioned medium + 75% KSR-FF
5. Passage 4 & thereafter: 100% KSR-FF

Note: If iPS cell lines are extremely problematic, a further level of caution can be taken by maintaining a culture in each prior passage medium while starting the next level of adaptation. For example, when passaging the 25/75 MEF-condtioned medium / KSR-FF culture (as described above), iPS cells can be passaged into both 100% KSR-FF AND 25/75 medium. If the 100% culture does poorly, adaptation can be resumed using the backup 25/75 culture.

Direct and partial sequential strategy:

6. Coat plates with Geltrex hESC-Qualified Reduced Growth Factor Basement Matrix as described above
7. Split cells into three plates (Plate 1, 2 & 3)
8. At the first passage, seed Plate 1 directly into KSR-FF and Plates 2 & 3 into the MEF-condtioned medium / KSR-FF culture.
9. On the next day, fluid-change Plate 2 with KSR-FF that day, and every day thereafter.
10. For Plate 3 at the second passage, try plating these cells directly into KSR-FF at a 1:2 split ratio (as above).