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DMEM:Ham's F12 (1:1)□w: 3.1 g/L□□□□□□w: 2.5 mM L-□□□□□□w: 15 mM HEPES□w: 0.5 mM□□□□□□□□□□□w: 1.2 g/L NaHCO3 (Cytion□□□□820400a)

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Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
Medium
HITES medium supplemented with 5% fetal bovine serum: The base medium for this cell line is DMEM:F12 Medium
(Catalog No. 820400a). To make the complete growth medium, add the following components to the base medium:
0.005 mg/ml Insulin
0.01 mg/ml Transferrin
30 nM Sodium selenite (final conc.)
10 nM Hydrocortisone (final conc.)
10 nM beta-estradiol (final conc.)
extra 2 mM L-glutamine (for final conc. of 4.5 mM)
5% fetal bovine serum (final conc.)
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

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