Data Sheet

BCRJ Code:	0256
Cell Line:	ZFL [ZF-L]
Species:	Danio rerio
Vulgar Name:	Zebrafish
Tissue:	Liver
Morphology:	Epithelial
Disease:	Normal
Growth Properties:	Adherent
Derivation:	The ZFL cell line was established in 1992 from a pool of approximately 10 normal adult zebrafish livers. It exhibits some properties characteristic of liver parenchymal cells.
Applications:	The cells are useful for studies of liver cell metabolism and xenobiotic formation. They may be used for in vitro toxicology studies. (The ZFL cell line was established in 1992 from a pool of approximately 10 normal adult zebrafish livers. It exhibits some properties characteristic of liver parenchymal cells).
Biosafety:	1
Culture Medium:	These cells are grown in 50% L-15; 35% DMEM High Glucose, 15% Ham's F12 - All without sodium bicarbonate Supplemented with: 0.01mg/ml bovine insulin, 50 ng/ml mouse EGF, 5% of heat-inactivated fetal bovine serum. NOTE: Do not filter complete medium.

Subculturing:	Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum that contains trypsin inhibitor. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping, do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. Add 6.0 to 8.0 mL of complete growth medium containing 10% heat-inactivated FBS and aspirate cells by pipetting gently. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant and resuspend cells in fresh serum-free growth medium. Add appropriate aliquots of cell suspension to new culture vessels. Place culture vessels in incubators at 28°C for 30 minutes. Examine to ensure attachment, and then add heat-inactivated FBS for a final concentration of 5%. Population Doubling Time: 72 hours NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.
Subculturing Medium Renewal:	Every 2 to 3 days
Subculturing Subcultivation Ratio:	1:2 to 1:3
Culture Conditions:	Atmosphere: air, 100% Temperature: 28°C; (Max. 29 °C, Min. 26°C)
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:	SAFETY PRECAUTION: Is highly recommend that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris. 1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the Oring and cap out of the water. Thawing should be rapid (approximately 2 minutes). 2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions. 3. For cells that are sensitive to DMSO is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes. 4.Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 5. Incubate the culture in a appropriate atmosphere and temperature (see "Culture Conditions" for this cell line). NOTE: It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
References:	53447: Ghosh C , et al. Derivation and characterization of a zebrafish liver cell line. Cell Biol. Toxicol. 10: 167-176, 1994. PubMed: 7994634 53448: Miranda CL , et al. Regulation of cytochrome P450 expression in a novel liver cell line from zebrafish (B
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