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: It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris. 1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes). 2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions. 3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately 125 × g for 5 to 7 minutes. 4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio). 5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line). NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at 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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