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BCRJ Code: 0094

Cell Line: GRX

Species: Mus musculus

Vulgar Name: Mouse; C3H/He

Tissue: Liver

Cell Type: Fibroblast

Morphology: Fibroblast

Disease: **Schistomiasis**

Growth Properties: Adherent

GRX cell line was established from primary cultures obtained by spontaneous **Derivation:** migration of cells from granulomas induced in liver of C3H/Hej mice infected

with Schistosoma mansoni.

Products: Collagen; fibronectin; laminin; heparan sulfate; chondroitin sulfate

Biosafety: 1

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These cells display myofibroblast characteristics, and are considered to represent murine liver cconnective tissue. They can be induced in vitro to display a fat-**Addtional Info:** storing phenotype corresponding to Ito cells by treatment with retinol, insulin, and indomethacin. GRX cells produce viral particles of the retrovirus type, are

anchorage dependent and have low contact inhibition.

Dulbecco's modified Eagle's medium with 4.5 g/L glucose and 10% of fetal bovine **Culture Medium:**

serum.

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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. T-75 flasks are recommended for subculturing this product. 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 and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. 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:

1:4 to 1:6 is recommended

Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C

Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

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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.

Thawing Frozen Cells:

- 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 $125 \times g$ 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:

In Vitro Cell. Develop. Biol. 21: 382-390, 1985; Biochim. Biophys. Acta 1011: 1-5, 1989; In Vitro cell. Develop. Biol. 26: 361-368, 1990. Schröder, S.K.; Schüler, H.M.; Petersen, K.V.; Tesauro, C.; Knudsen, B.R.; Pedersen, F.S.; Krus, F.; Buhl, E.M.; Roeb, E.; Roderfeld, M.; et al. Genetic and Molecular Characterization of the Immortalized Murine Hepatic Stellate Cell Line GRX. Cells 2022, 11, 1504. https://doi.org/10.3390/cells11091504

Characteristics: Established from fibrotic granulomas induced in C3H/HeN mice liver by experimental infection with Schistosoma mansoni (PubMed=2126187). Derived from site: In situ; Liver; Note=From a granulomatous inflammation; UBERON=UBERON_0002107. Cell type: Hepatic stellate cell; CL=CL_0000632.

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Cellosaurus: CVCL M115







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