

# PROTOCOL FOR SUBCULTURING AND FREEZING FIBROBLASTS

## Growth Media

DMEM - ThermoFisher #11960-044 (high glucose without L-glutamine)

15% FBS Fetal Bovine Serum – ThermoFisher #10437-028

1% (1X) Penicillin-Streptomycin - ThermoFisher #15140-122

1% (1X) GlutaMAX – ThermoFisher #35050-061

## Trypsin

Trypsin EDTA C 0.25% (ThermoFisher #25200-056)

## Hank's Balanced Salt Solution

HBSS- (ThermoFisher #14170 (1X) (-) calcium chloride, (-) magnesium chloride, (-) magnesium sulfate

## DMSO for Freezing

Cell Culture Grade DMSO - Sigma #D2438

## Thawing PRF cell lines

- 1) Thaw cells rapidly in a 37°C water bath.
- 2) Wipe the outside of the vial with 70% ethanol.
- 3) Transfer the thawed cells to a T25 flask containing 5 ml of growth media.
- 4) Place flask in 37°C incubator overnight.
- 5) The following day, observe under the microscope to ensure cells have attached.
- 6) Remove media and replace with fresh growth media.

## Subculturing PRF cell lines

- 1) Cells should be split when confluent.
- 2) To split cells (volumes given are assuming cells are in a T25):
  - a) Using sterile technique, remove media and rinse flask with 2-3 ml of sterile HBSS. Remove and discard HBSS.
  - b) Add 1 ml trypsin and incubate for 2-3 minutes. Cells should be checked under an inverted microscope to determine if they have begun to round up, lift and float. If cells are not detached after 3 minutes, you may incubate another 1-2 minutes.
  - c) Tighten cap and gently tip flask from side to side to dislodge all cells. Flask may be tapped lightly on side to loosen cells if needed.
  - d) Add 4 ml of new media to flask as soon as cells are floating to inactivate the trypsin. Rinse down sides of flask several times with the new media to wash all cells off the plastic and into the solution. Remove all liquid containing cells and transfer to a 15ml conical (or 50 ml if you are pooling 3 or more flasks).
  - e) Using sterile technique, remove an aliquot for counting on a hemocytometer.
  - f) While you are counting cells, the rest of the solution should be spun in the clinical centrifuge for 5 minutes at 1000 rpm.
- 3) After calculating your cell count, plate cells at  $2.5 \times 10^5$  cells per T25 filter top flask.
- 4) Cells should be fed every 2-3 days with fresh growth medium.

## Freezing:

Cells should be frozen at no less than  $5 \times 10^5$  cells/ml/cryovial in growth media containing 10% DMSO and 30% FBS and subsequently placed in an isopropanol freezing chamber at -80°C overnight. Transfer to the liquid nitrogen the next day.

**For Further Information Please Contact:**

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