Myofibroblast Isolation Protocol

- Use biopsies of lung tissue (4-7 sections of 2-3mm).

  Transfer fragments to a 50 ml tube.

  Add **HBSS (full)** and **300 U/ml antibiotics/antimycotic** solution.

- Rinse the samples with 25 ml **HBSS (full)** and **300 U/ml antibiotics/antimycotic** solution for *3-5 min*. Mix gently.

  Place the tube horizontally for 3 min at room temperature. Allow tissue to settle and discard supernatant.

  Repeat x 3.

  After the second rinse we transfer samples to a fresh 50 ml tube. After the third rinse, centrifuge at 200 x g for 10 min.

  Discard supernatant.

- Rinse samples for *3-5 min* with 25 ml **HBSS (without Ca and Mg)** and **300 U/ml antibiotics/antimycotic** solution.

  Place the tube horizontally for *3 min* at room temperature.

  Allow tissue to settle down and discard supernatant.

  Repeat x 3.

  After the second rinse, we transfer samples to a fresh tube.

  After the third rinse, centrifuge at 200 g for *10 min*.

  Discard the supernatant.

- Rinse the tissue with 20 ml HBSS (without Ca and Mg) and 300 U/ml antibiotics/antimycotic solution and 1mM DTT (57µl DTT at 20 ml), mix gently.

  Place the tube horizontally (15 min at room temperature) and mix gently every 5 min.

- Rinse the tissue with 20 ml **HBSS (without Ca and Mg)** and **300 U/ml antibiotics/antimycotic solution**, mix gently.

  Place the tube horizontally for *3 min* at room temperature.

  Allow tissue to settle down and discard supernatant.

  Repeat x 3.

After the second rinse we transfer the samples to a fresh tube. After the third rinse, centrifuge at 200 x g for *10 min*.

Discard the supernatant.

- Incubate at 37 °C with **HBSS (without Ca and Mg)** and **300 U/ml antibiotics/antimycotic** solution and **1mM EDTA** for *30 min.*

  Vortex for *2 min* every 10 min.

  Allow tissue to settle down and discard supernatant.

  Rinse with 25 ml **HBSS (without Ca and Mg)** and **300 U/ml antibiotics/antimycotic** solution, mix gently.

  Allow tissue to settle and discard supernatant.

  Repeat x 3.

- After the third rinse add 10 ml **RPMI**.

  Mix gently and allow tissue to settle down.

  Discard the supernatant.

- Transfer the tissue to flask 75 cm^2 and add 12 ml **RPMI**
- Transfer the flask to the incubator at 37 °C and 5% CO_2.

Notes :

- Mediums should be at room temperature 10 min before use.
- Prepare 3 x 50 ml conical tubes with 25 ml HBSS + EDTA at 37 °C 10 min before use in step 7.
- HBSS (full) and HBSS (without Ca and Mg) → **Hank's Balanced Salt Solution** is used for a variety of cell culture applications, such as washing cells before dissociation, transporting cells or tissue, and preparing reagents. Formulations without calcium and magnesium are required for rinsing chelators from the culture before cell dissociation.
- Antibiotics/antimycotic → penicillin, streptomycin / amphotericin
- DTT → **Dithiotreitol**
- EDTA →**Ethylenediaminetetracetic Acid** dissolves lime scale.
- RPMI →bicarbonate buffering system
- FBS 10x → **Fetal Bovine Serum** allows the cells to acclimatize to the new material.
- Cells may take 2-6 weeks to become confluent for the first time but will usually grow quicker after that.

- After a few weeks, if colonies have appeared but are not spreading out very well, cells can be trypsinized to promote growth. They can be left in the same flask or moved to a new flask if there are not enough cells to split.
- Begin freezing stocks of cells at passages 2-6. Cells should be used for assays ideally between passages 3 and 5. Sometimes cells just stop growing around passages 2-5.