Subculturing Adherent Cells – SPLIT

The following protocol describes a general procedure for subculturing adherent cells in culture**.**

- Remove and discard the spent cell culture media (DMEM) from the flask.
- Wash cells using 15 ml **PBS 1x.** Gently add PBS to the side of the vessel opposite the attached cell layer to avoid disturbing the cell layer, and rock the vessel back and forth several times. Repeat x 3.
- Remove and discard the PBS from the flask.
- Add 3ml of pre-warmed (37 °C) **trypsin EDTA** to the side of the flask. Use enough reagent to cover the cell layer. Gently rock the container to get complete coverage of the cell layer.
- Discard 2 ml of trypsin EDTA and transfer the flask into the incubator for *5 min.*
- Observe the cells under the microscope for detachment. Tap the flask to expedite cell detachment before observation.
- Add 10 ml of pre-warmed complete growth medium (**DMEM**) to stop the reaction. Disperse the medium by pipetting over the cell layer surface several times.
- Transfer the cells to a 15-mL conical tube and centrifuge then at 200 x g for 10 min.
- Discard the DMEM and add 11 ml fresh.
- Pipet the appropriate volume into new cell culture vessels (e.g. 6 well plates), and return the cells to the incubator or follow the procedure of freezing.

Notes :

1. PBS 1x → 5 ml PBS 10x in 45 ml dH_2O.
   PBS is a balanced salt solution without calcium and magnesium that removes any traces of serum, calcium, and magnesium that would inhibit the action of the dissociation reagent (trypsin).
2. Trypsin EDTA → Toxic! (discard 2 ml – step 5)
   Freezing → room temperature – 10 min → incubator – 10 min.
3. Cells need 24-48 h to attach to the flask.
4. Every 24-48 h DMEM should be replaced with fresh.
5. **Avoid** culture to come in contact with the flask cap.