**Extraction Buffer:**

Dulbecco’s phosphate Phosphate-buffered saline, Ca++ and Mg++ free

0.5% (v/v) Triton X-100

20 mM NH4OH

Store up to 1 month at 4°C

**Culturing Cells on Glass Coverslips for ECM Growth**

IMR-90 Cells are grown for 7-10 days on glass coverslips coated with FN.

The surface of the plastic cover dishes is blocked using PMMA overnight using a small amount of solution.

FN is adsorbed to the surface overnight at 4oC at a concentration of 1ug/cm2 as calculated for the entire well.

UV light is used to treat the FN coated coverslips for 45 minutes prior to incubation in the refrigerator.

**Removing cells from ECM**

Remove coverslips from the 12 well plate into a new dry 12 well plate first.

Carefully aspirate the medium and rinse gently two times with 2 ml DPBS - touching the pipet against the dish wall rather than at the bottom of the dish.

Gently add 1 ml of pre-warmed (37°C) extraction buffer.

Observe the process of cell lysis using an inverted microscope. Incubate at 37oC until no intact cells are visualized (~5-10 min).

If necessary, re-wash with 1x PBS and perform the procedure again. It is possible that other additives may be necessary for optimization of the procedure, such as EDTA (to the extraction buffer) to remove more cells or citric acid (to the cell media).

Once done, rinse the coverslips in 1x PBS and leave in PBS for 3 days to help the matrix adhere to the glass better. Some cells may still be adhering and they will die if left in 1x PBS at 4oC for prolonged periods.

DNA will still remain on the ECM in many cases, even after several days in PBS. You may remove the remaining DNA using Pierce Universal Nuclease at the manufacturer recommended concentration for the volume of the well at 37oC for 45 minutes.

**Procedure for antibody labeling of ECM**

Warm PBS + 4% Formaldehyde in PBS

Wash 3x with PBS

Fix cells with 4% Formaldehyde in PBS for 10 minutes

Wash with 3x with 1x PBS

Permeabilize cells with TBS-T for 10 minutes

Rinse with Abdil (2% BSA in TBST) for 20 minutes

Add primary antibodies at a dilution depending on what you want to label (in order) in a solution of Abdil to prevent non-specific adhesion.

Add laminin primary antibody (1:100) for 1 hr

Wash 3x with TBS-T

Add 2o antibody (rabbit for laminin) 1h at room temperature

Wash TBS-T