

## **Cell-Matrix Adhesion Assay General protocol Stack Lab**

### 1. Coat plates:

- a. We use 24 wells plates. Dissolve proteins in coating buffer (0.1 M Sodium Carbonate pH 9.6) to a final concentration of 10 ug/ml.
- b. Add 300 ul/ well and incubate at 4°C O/N or 37°C two hours.
- c. Wash coated wells once with PBS.
- d. Let dish air dry in hood, store at 4°C wrapped in Parafilm for up to 6 months.

2. Block non-specific binding by incubating plate with 500 ul of 3% BSA in the appropriate serum free cell culture medium (SFM) for 1 hour in 37°C while preparing cells. Remove solution before use.

### 3. Prepare cells:

- a. Wash the cells 3 times with PBS.
- b. Trypsinize the cells, neutralize trypsin with Soybean Trypsin Inhibitor.
- c. Resuspend the cell in SFM
- d. Count the cells and dilute to a final concentration of 100,000/ml.

### 4. Seed cells

- a. Set up at least duplicate wells for each condition; add 500 ul cells to each well.
- b. Incubate at 37°C (in tissue culture incubator) for 15- 30 min. Adhesion time depends on the cell line. It is useful to do a time course first, choosing several time points between 5-10 min and 2 h

### 5. Stop the assay:

- a. Rinse cells once with PBS
- b. Fix cells with 3.7% Formaldehyde for 10 min. or use Diff-quick solutions.

### 6. Count the cells

See file "Cell Counting Migration Invasion Assays" for cell counting protocol