

## Adhesion Assay Protocol:

### Materials to be prepared beforehand:

- 1) Washing Buffer--0.1% BSA in medium (DMEM or RPMI)
- 2) Blocking Buffer--0.5% BSA in medium (DMEM or RPMI)
- 3) Laminin-1 10-12  $\mu\text{g/ml}$  or FN 20  $\mu\text{g/ml}$
- 4) 96-well-plate
- 5) Crystal violet (5mg/ml in 2% Ethanol)
- 6) 1% SDS in H<sub>2</sub>O
- 7) 4% paraformaldehyde

### Procedures:

- 1) Coat 96-well-plate with Laminin-1 or FN at 37 °C for 1 hr or at 4 °C O/N. Leave some wells uncoated as negative control.
- 2) Wash with washing buffer for 2 times.
- 3) Block plates with blocking buffer at 37 °C in CO<sub>2</sub> incubator for 45-60 minutes.
- 4) Wash with washing buffer.
- 5) Chill the plates on ice.
- 6) Count cell to 4 X 10<sup>5</sup>/ml. Add 50  $\mu\text{l}$  cells in each well.
- 7) Incubate in CO<sub>2</sub> incubator at 37 °C for 30 minutes.
- 8) Shake the plate at 2000 rpm for 10-15 seconds. Wash with washing buffer 2-3 times.
- 9) Fix with 4% paraformaldehyde. Incubate at RT for 10-15 minutes.
- 10) Wash with washing buffer.
- 11) Stain with Crystal Violet for 10 minutes.
- 12) Wash with water.
- 13) Turn the plates upside down. Let the plates dry up completely.
- 14) Add 2% SDS. Incubate at RT for 30 min.
- 15) Read plate at 550 $\mu\text{m}$ .