Human Fibronectin (FN) Monoclonal Antibody (Cat.# M002 and M010)

## Application: Assessing fibronectin-mediated cell adhesion using anti-fibronectin antibodies

Fibronectin (FN) is glycoprotein component of the extracellular matrix that plays a role in cell adhesion, growth, and migration. In this experiment, monoclonal neutralizing antibodies specific to FN (Cat.# M002 and M010) were used to assess the fibronectin-mediated adhesion of normal rat kidney (NRK) cells.

## **Methods**

To assess fibronectin-mediated cell adhesion, anti-fibronectin antibodies were added to wells of a fibronectin-coated plate prior to the addition of NRK cells (Figure 1). The number of attached cells was estimated by rose bengal staining and microscopic observation.

Coat 96-well microplate with FN (10 µg/ml in PBS)

Block with 2% BSA in PBS

Incubate with monoclonal FN antibodies

(Cat.# M002 and/or M010)

Add trypsinized NRK cells suspended serum-free DMEM

(10,000 cells per well) and incubate for several hours

Wash with PBS

Wash with PBS

Wash with PBS

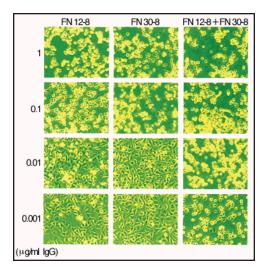
Treat with 50% ethanol in PBS

Estimate the number of attached cells by microscopy

**Figure 1.** Overview of the experimental procedure for assessing fibronectin-mediated cell adhesion of NRK cells.

## Results

Pretreatment of FN-coated plates with antibodies that recognize the cell-binding domain of FN reduced cell adhesion as assessed by cell staining and microscopy. The effect was dose dependent as fewer adherent cells were observed when the FN antibody concentration increased (Fig. 2).



**Figure 2.** NRK cell adhesion. Various concentrations (0.001–1  $\mu$ g/ml) of monoclonal FN antibodies (Clone 12-8, Cat.# M002; Clone 30-8, Cat.# M010), alone or in combination, were used to assess adhesion of NRK cells through interaction with FN. After removal of non-adherent cells, cells were visualized by rose bengal staining and microscopy.

## **Conclusions**

Both clones (FN 12-8 and FN 30-8) recognize the cell-binding doman of FN. Pretreatment with either monoclonal FN antibody (Cat.# M002 or M010) interfered with FN-mediated NRK cell adhesion. Combined use of both antibody clones caused an increased inhibition of cell adhesion.