Bovine Fibronectin Coated 96-Well Microplates

ORDERING INFORMATION & SPECIFICATIONS

Catalog Number: CWP002

Size: 5 Coated Tissue Culture Microplates

Storage: 2 - 8° C*

Coating Protein: Bovine Fibronectin

Concentration of Coating Protein: 1.0 µg/well

Coating Volume: 100 µL/well

Blocking Protein: BSA

Blocking Volume: 200 µL/well

Related Products: Visit www.RnDSystems.com for other coated microplates

Product Description

Bovine fibronectin coated 96-well microplates are clear, polystyrene tissue culture microplates coated with 0.2 micron filtered serum derived bovine fibronectin and /or blocked with Bovine Serum Albumin Fraction V under aseptic conditions.

Intended Use

These plates may be used as a solid support for cell attachment of adherent cell lines. An example protocol for a cell adhesion assay is described below. Results of a typical adhesion assay using these plates are shown in Figure 1.

Other Supplies Required

- Pipettes and disposable pipette tips
- Calcein AM Solution (Molecular Probes, Catalog # C3099 or equivalent)
- Fluorescence reader capable of measuring fluorescence in a 96-well microplate with filter set at 485 nm wavelength for excitation and 520 nm wavelength emission
- Dulbecco's PBS with calcium and magnesium (GIBCO®, Catalog # 14040-117 or equivalent)
- Microplate centrifuge
- 37° C incubator

Protocol for a Cell Adhesion Assay using Bovine Fibronectin 96-Well Microplates

Notes: *Remove all air bubbles from wells before using a fluorescence plate reader.*

Optimal cell number per well will be dependent on cell size and should be determined by each laboratory for each cell type. For most applications, the goal should be to add the maximal number of cells per well without the cells coming into contact with one another.

Optimal incubation time for maximal adhesion should be determined by each laboratory for each cell type.

Optimal number of washes for each cell type should be determined by each laboratory.

1. Harvest cells and then resuspend the cell pellet in PBS with calcium and magnesium at a concentration of 2.5 x 10^6 cells/mL.
2. Prepare a 1 mM working solution of calcein AM according to the manufacturer's instructions. Add calcein AM to cells at a final concentration of 12.5 µM.
3. Incubate cells at room temperature for 30 minutes.
4. Wash the cells twice in PBS with calcium and magnesium.
5. Resuspend the cells in PBS with calcium and magnesium at a concentration of 1 x 10^6 cells/mL.
6. Add 100 µL of prepared cell suspension to each well in triplicate. Spin the plates at 411 x g for 2 minutes in order to move the cells to the plate surface.
7. Incubate plates for 30 minutes at 37° C.
8. Measure the "before wash" fluorescence of the samples using a fluorescence plate reader at excitation wavelength 485 nm and emission wavelength 520 nm.
9. Wash away non-adherent cells using the following procedure:
   a) Invert the plate and tap out contents of the wells.
   b) Add 200 µL/well of 1X PBS, rotate the plate horizontally 180°, invert the plate, and tap out the contents of the wells.
   c) Repeat step b.
   d) Add 100 µL/well of 1X PBS to all wells.
10. Read plate "after wash" on the fluorescence plate reader.
11. Calculate the average percent adhesion using the following formula: $[(RFU_{\text{after wash}})/(RFU_{\text{before wash}})] \times 100$
12. Typical results are represented in Figure 1 using three different cell lines.

**Unopened plates may be stored at 2 - 8° C until the expiration date on the label. It is recommended that all wells in each plate be used at the same time. Half-used plates should be discarded.*

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

R&D Systems, Inc.
1-800-343-7475

Assay Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA
B	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN
C	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN
D	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN
E	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN
F	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN
G	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN	FN
H	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA

BSA = Bovine Serum Albumin Fraction V
FN = Fibronectin

Example

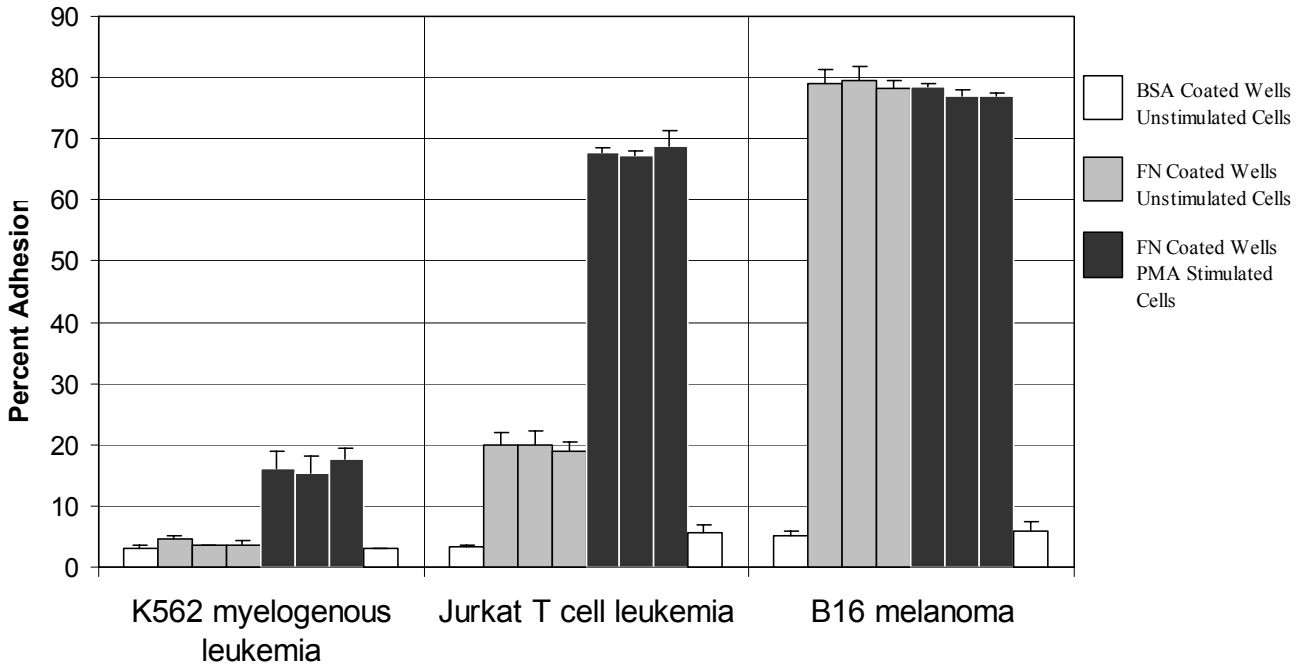

Figure 1: Adhesion of calcein AM labled cells to R&D Systems Bovine Fibronectin coated microplates. Adhesion of the cells to BSA (open bars; shown as a triplicate in Row A and Row H), to fibronectin (FN) in the absence of stimulation (gray bars; shown as a triplicate in Rows B - D), and to fibronectin in the presence of 10 ng/mL PMA (black bars; shown as a triplicate in Rows E - G) was determined after a 30 minute incubation.