Fibronectin fragment, premium grade

Cat. No. FIN-H5113 **Size: 500 μg**

Description

Fibronectin is a large modular glycoprotein and a ligand for many molecules including fibrin, heparin, chondroitin sulfate, collagen/gelatin, and integrins. It is involved in multiple cellular processes such as cell adhesion/migration, blood clotting, morphogenesis, tissue repair, and cell signaling. Recombinant fibronectin fragment contains three functional domains: the cell-binding domain (C-domain), heparin-binding virus adhesion domain (H-domain), and CS-1 sequence. The fragment can enhance retroviral-mediated gene transduction by aiding the co-localization of target cells and virions. The viral particle can bind to the virus adhesion area (H-domain) of fibronectin, and target cells bind mainly through the interaction of cell surface integrin receptor VLA-5 and VLA-4 with the fibronectin C-domain and CS-1 site, respectively. So that it can close to the cells and viruses, to improve the efficiency of virus infection. When fibronectin fragment is coated on the surface of cell culture dish, it significantly enhances retroviral-mediated gene transfer to target cells.

Formulation

Sterile solution containing 12.5 mM sodium citrate (pH 6.2).

Materials Required but not Provided

[Equipment]
- Electric pipetter
- Pipetter
- Sterile pipettes
- Sterile tips with filters
- Safety cabinet or clean workstation
- Microscope
- CO_2 incubator
- Microplate centrifuge
- 24-well-plate (No Tissue culturetreated)

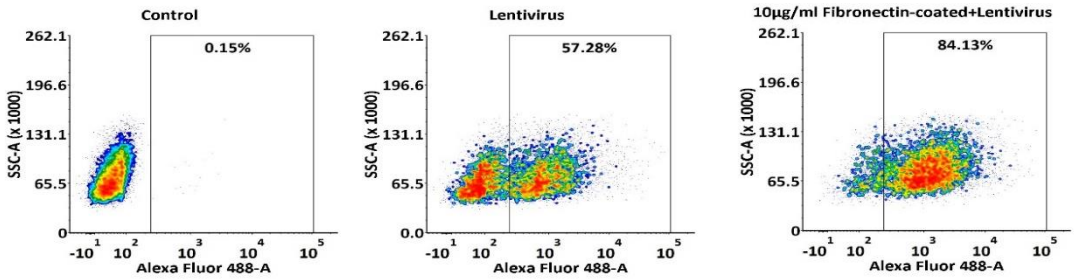
[Reagents]
- Jurkat cells
- RPMI medium
- pLenti-CMV-EGFP-puro-Amp, 4.40E+08 TU/ml (Acro)
- Fetal bovine serum
- FACS buffer: 2% BSA in PBS, pH7.2-7.4

Reference Protocol

1) Dilute the sample by PBS, the final concentrations is 10 μg/ml.
2) Add 500 μl of sample solution diluted by PBS to 24-well-plate (No Tissue culturetreated), and place 24-well-plate at 4℃ overnight.
3) Remove the coated sample solution, add 500 μl of PBS containing 2%BSA to 24-well-plate, and place 24-well-plate at room temperature for 30 min.
4) Remove PBS containing 2%BSA, and wash 24-well-plate twice with PBS.
5) Adjust the cell density to $2*10^5$ cells/ml and seed the cells at $2*10^5$ cells/well in 24-well-plate.

6) Calculate the used volume of lentivirus, add the lentivirus to the cells, and incubate the cells at 37℃, 5% CO_2 for 48 hrs.
7) Wash the cells twice with FACS buffer and resuspend the cells in PBS.
8) Detect the fluorescence (GFP) using Flow cytometry.
9) Analyze the data using FCS Express 7 software.

Typical Data



2e5 of Jurkat cells were transfected with pLenti-CMV-EGFP-puro-Amp for 48hrs in the presence or absence of Fibronectin fragment, premium grade (Cat. No. FIN-H5113)-Coated. The fluorescence of GFP were detected with FACS, Alexa Fluor 488 signal was used to evaluate the expression of GFP+ Jurkat cells (Routinely tested).