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Product Information

Fibronectin FITC

Product Number **F2733**

Product Description

Fibronectin is purified from human plasma (**Cat. No. F0895**). The protein is modified to contain covalently linked Fluorescein 5-isothiocyanate (**Cat. No. F7250**). Fibronectin, also known as Cold-insoluble globulin, is a large glycoprotein of the extracellular matrix that is coded by FN1 gene. It is expressed in the plasma and at the cell surface. Fibronectin is a ubiquitous and essential component of the extracellular matrix (ECM) and plays a vital role during tissue repair[1]. Fibronectin functions both as a regulator of cellular processes and an important scaffolding protein to maintain and direct tissue organization and ECM composition[1]. Fibronectin plays an important role in cell adhesion and spreading and affect the routes of cell migration both in vivo and in culture[2].

Proteolytic degradation of extracellular matrix (ECM) is a critical step during cell invasion and tissue transmigration that is required for many physiological and pathological processes. Cellular structures that mediate cell adhesion to, degradation of, and invasion into ECM are invadopodia of transformed and tumor cells and podosomes of osteoclasts, macrophages, normal monocytic, endothelial, and smooth muscle cells [3, 4, 5]. The ability to degrade extracellular matrix (ECM) is a hallmark of invasive tumors and is thought to be essential for the movement of cancer cells through tissue barriers.

The invadopodia assay is a method that has been most informative for pinpointing regions of the cell that initiate invasion involve plating cells on a culture surface coated with a thin layer of fluorescently labeled matrix, and visualizing regions where the cell has degraded the matrix to create an area devoid of fluorescence[6].The assay have revealed that invasive cells extend small localized protrusions that degrade the matrix. This invadopodia invasion assay may be used for assessing activity of different cell types as well as individual cells in heterogeneous populations may be analyzed for invasive potential[7]. The number and invadopodia activity are sensitive to some physical or chemical factors such as: cell type, matrix rigidity, density of cell layer [3,6, 8, 9].

Reagent

Supplied as a solution in 0.05 M TRIS Buffered solution pH 8.0 with 0.05% MIT as a preservative. It is sterile-filtered using a 0.2 um filter.
Fibronectin Concentration: ~ 1.0 mg/mL

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store unused portion in working aliquots at -20°C. Repeated freezing and thawing is not recommended.

Preparation Instruction

The solution should be thawed slowly at 2-8 °C and before use heated at 37 °C.

The Fibronectin-FITC is suitable for invadopodia assay. 30-60 µg of Fibronectin-FITC is required to coat one 12-mm Diameter round glass slide for a single FITC-Fibronectin degradation assay.

Procedure

Note: In order to obtain best results in different techniques/preparations and sampling strategies must be determined by the end user.

Invadopodia invasion cell test.

Assay is adapted for using on 12mm glass coverslip as substrate carrier. Make sure that each slide coated by liquids during each step of assay.

1. Put the glass slide to well of Costar 24-well plate for test.
2. Add 50 ug/ml poly-L-lysine (**Cat. No. P1524**) solution to well. Incubate at room temperature for

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20 minutes. Accurately remove poly-L-lysine solution; rinse three times with sterile PBS.

3. Add 0.5% Glutaraldehyde (**Cat. No. G7651**) solution to well. Incubate at room temperature for 15 minutes. Accurately remove Glutaraldehyde solution; rinse three times with sterile PBS.
4. Prepare Gelatin solution(Cat. No. **G2500**) at 37°C.
5. Pre-heat Fibronectin-FITC at 37°C for 15 minutes.
6. Add 30-60 µg Fibronectin FITC to Gelatin solution: the final concentration in assay is 0.5% Gelatin. Store mix at 37°C. Protect from light.
7. Add the Fibronectin-FITC /gelatin mix solution to well. Incubate at 37°C for 10 minutes, protect from light. Gently remove mix solution; rinse three times with sterile PBS
8. For substrates disinfection- incubate with 70% ethanol solution at room temperature for 30 minutes, protect from light. Gently remove 70% ethanol solution; rinse three times with sterile PBS, protect from light.
9. Add growth media with fetal bovine serum and incubate at room temperature for 30 minutes, protect from light.
10. Remove growth media and seed cells in fresh growth media, protect from light.
11. Place chamber slides into a 5% CO_2 tissue culture incubator or at 37°C for the desired culture duration. Protect chamber from light.

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Troubleshooting Guide

Problem	Possible Solutions
Undesirable coated matrix quality (signal intensity, homogeneity)	• Initial quality of glass surface may affect quality of coated matrix – consider glass cleaning step (e.g., acid wash) prior to poly-L-lysine coating, assuming wash compatibility will all materials associated with substrate (e.g. well material for chamber slide). • Glass chamber slides and dishes/multi-well plates may exhibit batch-to-batch variability in ability to support homogeneous matrix coating – try alternate batch of glass surface. • Adjust amount of FITC-Fibronectin in mixture (i.e., ratio of labeled protein : unlabeled gelatin) to increase or decrease signal intensity from matrix, as appropriate for application of interest. • The store temperature at 37^{O}C is a critical for Fibronectin FITC /gelatin mix solution homogeneity adherence the protein substrate on the glass (Steps 5-7). • Optimize imaging conditions (magnification, numerical aperture, exposure time, gain, illumination intensity) for enhanced signal.
Insufficient or excessive substrate matrix degradation	• Degree or presence of degradation can be highly cell type-dependent, cell's layout density, growing medium and/or growing conditions. • Cell lines can consist of subpopulations with different degrees of invadopodia formation, and the proportions of these subpopulations may change over time in continuous culture. Use each cell line at a defined passage number, and/or early passage cells from the same bank of cells, for best consistency between experiments. • Perform time-course of substrate degradation for cell type of interest. • Degree of substrate degradation may be influenced by glass properties – test alternate glass formats or products. • Percent of final gelatin concentration in substrate matrix may affect on type and form of invadopodia formation.

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Poor fluorescence quality (signal intensity, background)	• Increase concentration of FITC-Fibronectin in substrate mix solution. • Increase concentration of serum to quench residual free aldehydes in (Step9). • Protect fluorescent content samples from light at all times. • Failure to maintain sterility during process –use DAPI counterstaining for fixed cells. • Optimize imaging conditions (magnification, exposure time, gain, illumination intensity) for enhanced signal.

References

1.To W.S., Midwood K.S. (2011) Plasma and cellular fibronectin: Distinct and independent functions during tissue repair. Fibrogenesis Tissue Repair., **4**: 21.
2.Ruoslahti, E. (1984). Fibronectin in cell adhesion and invasion. Cancer Metastasis Rev., **3:** 43-51
3.Artym VV, Yamada KM and Mueller SC (2009) ECM degradation assays for analyzing local cell invasion. Methods Mol Biol **522**:211-219.
4.Murphy DA, Courtneidge SA (2011) The 'ins' and 'outs' of podosomes and invadopodia: characteristics, formation and function. Nat Rev Mol Cell Biol **12**: 413–426.
5.Weaver AM. (2006) Invadopodia: specialized cell structures for cancer invasion. *Clin Exp Metastasis*. **23**: 97–105.
6.Chen WT, Olden K, Bernard BA and Chu FF (1984) Expression of transformation-associated protease(s) that degrade fibronectin at cell contact sites. J Cell Biol **98**:1546-1555.
7.Wang Z, Liang X, Cai M, Du G (2016). Analysis of invadopodia formation in breast cancer cells. Methods Mol Biol **1406**: 203-210.
8.Alexander NR, Branch KM, Parekh A, Clark ES, Iwueke IC, Guelcher SA, Weaver AM. (2008) Extracellular Matrix Rigidity Promotes Invadopodia Activity. Curr. Biol. **18**:1295-9.
9.Weaver AM., Parekh A. (2009) Regulation of cancer invasiveness by the physical extracellular matrix environment. J. Cell adhesion and migration. **3:** 288-292.

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