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## 1. Description

**Products** Human Fibronectin (Fragment), premium grade.  
Recombinant engineered human fibronectin fragment for use in cell culture.

Content in µg	Order no.
100	130-109-392
1000	130-109-393

**Biological activity** The ED<sub>50</sub> is ≤5000 ng/mL. Lot-specific activities are stated in the Certificate of Analysis ([www.miltenyibiotec.com/certificates](http://www.miltenyibiotec.com/certificates)).

▲ **Note:** The ED<sub>50</sub> is determined by an adhesion assay using BHK-21 cells according to Shimizu *et al.*<sup>1</sup> The wells of a 96-well plate were coated using 0.045–100 µg/mL human Fibronectin (fragment) with a volume of 50 µL for each well (0.32 cm<sup>2</sup>).

**Primary structure** Single glycosylated polypeptide chain (459 amino acid residues).

**Molecular mass** 49.9 kDa (monomer).

**Source** Produced in *Pichia pastoris*.

**Product format** Lyophilized from a filtered (0.2 µm) buffer solution.

**Stabilizer** Mannitol and trehalose.

**Purity** >95% as determined by SDS-PAGE analysis.

**Endotoxin level** Low endotoxin (<1 EU/µg cytokine) as determined by Limulus Amebocyte Lysate (LAL) assay.

**Storage** Lyophilized Human Fibronectin (Fragment), premium grade should be stored at –20 °C. The expiration date is indicated on the vial label. Upon reconstitution aliquots should be stored at –20 °C or below. Avoid repeated freeze-thaw cycles.

**Reconstitution** It is recommended to reconstitute lyophilized Human Fibronectin (Fragment), premium grade with sterile PBS buffer to a final concentration of 0.1–1.0 mg/mL. Further dilutions to a final concentration of 20–100 µg/mL should also be done with sterile PBS buffer immediately before usage. Do not filter the solution. For plate coatings, a minimum concentration of 20 µg/mL is recommended.

**Coating** The coating should be 3–5 µg of Human Fibronectin (Fragment) per cm<sup>2</sup>. For example, when coating a 35 mm diameter dish (9 cm<sup>2</sup>), use 1.35 mL of a 20 µg/mL solution for 3 µg/cm<sup>2</sup> or 2.25 mL for 5 µg/cm<sup>2</sup>. Dispense the appropriate volume of Human Fibronectin (Fragment) solution into each plate and incubate overnight at 4 °C. Remove the solution.

▲ **Note:** For cultivation of cardiomyocytes and endothelial cells, additional BSA blocking is not required.

### 1.1 Background information

Fibronectin is a high-molecular weight glycoprotein of the extracellular matrix. It is of major importance for cell adhesion, growth, migration, and differentiation. Fibronectin binds to extracellular matrix components, such as collagen, heparin sulfate proteoglycans or collagen. Furthermore, as part of the family of extracellular matrix proteins (ECM) it facilitates the delivery of growth factors to cells. Fibronectin has profound effects on wound healing, including the formation of proper substratum for migration and growth of cells during the development and organization of granulation tissue, as well as remodeling and resynthesis of the connective tissue matrix. The human Fibronectin (Fragment) is a recombinant engineered protein fragment composed of two functional domains of the full length protein.

### 1.2 Applications

Human Fibronectin (Fragment) may be used for a variety of applications, including:

- Culture of cardiomyocytes derived from pluripotent stem cells
- Culture of mesenchymal stem cells (MSC)
- Culture of isolated cardiomyocytes

Optimal concentration for a specific application should be determined by a dose-response experiment.

## 2. References

1. Shimizu, M. *et al.* (1997) Establishment of a standardized assay system of fibronectin activity using fibronectin-mediated cell adhesion. *Biol. Pharm. Bull.* 20 (12): 1219–1223.

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