abcam

Product datasheet

Human Fibronectin ELISA Kit ab219046

SimpleStep ELISA®

9 Images

Overview

Product name Human Fibronectin ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
Serum	3			5%

Inter-assay

Sample	n	Mean	SD	CV%
Serum	5			8.3%

Sample type Cell culture supernatant, Milk, Serum, Cell culture extracts, Tissue Extracts, EDTA Plasma, Citrate Plasma

Assay type Sandwich (quantitative)

Sensitivity 20.6 pg/ml

Range 125 pg/ml - 8000 pg/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Cell culture supernatant	102	90% - 117%
Milk	102	91% - 111%
Serum	93	87% - 102%
Cell culture extracts	89	80% - 105%
Tissue Extracts	90	80% - 131%
EDTA Plasma	86	81% - 94%

Sample type	Average %	Range
Citrate Plasma	99	87% - 120%

Assay time 1h 30m

Assay duration One step assay

Species reactivity

Reacts with: Mouse, Human, Rhesus monkey
Does not react with: Rat, Cow

Product overview

Human Fibronectin SimpleStep ELISA® kit **(ab181419) has been re-developed** with new capture and detector antibodies. This new kit has the same name but a different product number (ab219046). We have identified new recombinant monoclonal antibodies to use in the SimpleStep ELISA platform that provide a higher sensitivity when quantifying Fibronectin in cell culture extracts, citrate plasma, EDTA plasma, cell culture supernatant and tissue extracts.

Fibronectin *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Fibronectin protein in human serum, plasma, milk, cell culture supernatant, and cell and tissue extract samples.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Sensitivity:

Sample in Sample Diluent NS: 20.6 pg/mL

Samples in 1X Cell Extraction Buffer PTR: 22.3 pg/mL

Notes

Fibronectin is a large glycoprotein present in the extracellular matrix and circulating plasma. Fibronectin is important in many cell adhesion and migration related processes, including wound healing, embryogenesis and nerve regeneration. Differential expression of fibronectin is seen in coronary heart disease, glomerulopathy and tumor cell metastasis. The protein contains binding sites for collagen, heparin and fibrin and is a specific ligand for several integrin adhesion receptors. Fibronectin exists as a dimer or multimer.

Tested applications **Suitable for:** Sandwich ELISA

Platform Pre-coated microplate (12 x 8 well strips)

Properties

Storage instructions	Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Wash Buffer PT (ab206977)	1 x 20ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent 4BR	1 x 6ml
Human Fibronectin Capture Antibody 10X	1 x 600µl
Human Fibronectin Detector Antibody 10X	1 x 600µl
Human Fibronectin Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS	1 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

Function	Fibronectins bind cell surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin. Fibronectins are involved in cell adhesion, cell motility, opsonization, wound healing, and maintenance of cell shape. Involved in osteoblast compaction through the fibronectin fibrillogenesis cell-mediated matrix assembly process, essential for osteoblast mineralization. Participates in the regulation of type I collagen deposition by osteoblasts. Anastellin binds fibronectin and induces fibril formation. This fibronectin polymer, named superfibronectin, exhibits enhanced adhesive properties. Both anastellin and superfibronectin inhibit tumor growth, angiogenesis and metastasis. Anastellin activates p38 MAPK and inhibits lysophospholipid signaling.
Tissue specificity	Plasma FN (soluble dimeric form) is secreted by hepatocytes. Cellular FN (dimeric or cross-linked multimeric forms), made by fibroblasts, epithelial and other cell types, is deposited as fibrils in the extracellular matrix. Ugl-Y1, Ugl-Y2 and Ugl-Y3 are found in urine.
Involvement in disease	Glomerulopathy with fibronectin deposits 2
Sequence similarities	Contains 12 fibronectin type-I domains. Contains 2 fibronectin type-II domains. Contains 16 fibronectin type-III domains.
Developmental stage	Ugl-Y1, Ugl-Y2 and Ugl-Y3 are present in the urine from 0 to 17 years of age.
Post-translational modifications	Sulfated. It is not known whether both or only one of Thr-2064 and Thr-2065 are/is glycosylated. Forms covalent cross-links mediated by a transglutaminase, such as F13A or TGM2, between a glutamine and the epsilon-amino group of a lysine residue, forming homopolymers and heteropolymers (e.g. fibrinogen-fibronectin, collagen-fibronectin heteropolymers).

	Phosphorylated by FAM20C in the extracellular medium. Proteolytic processing produces the C-terminal NC1 peptide, anastellin.
Cellular localization	Secreted, extracellular space, extracellular matrix.

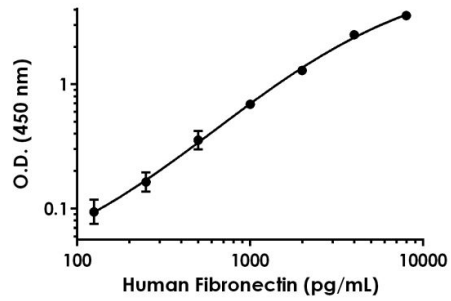
Applications

Our Abpromise guarantee covers the use of **ab219046** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

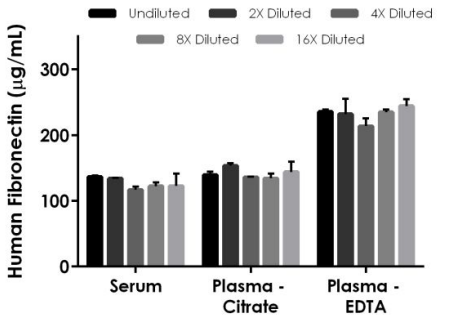
Application	Abreviews	Notes
Sandwich ELISA		Use at an assay dependent concentration.

Images



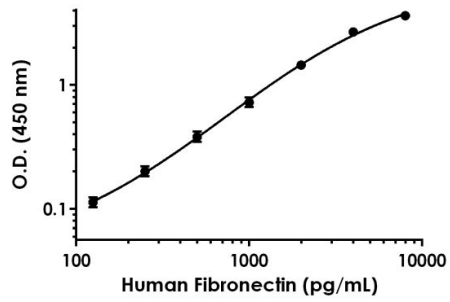
Example of human Fibronectin standard curve in Sample Diluent NS.

Background-subtracted data values (mean +/- SD) are graphed.



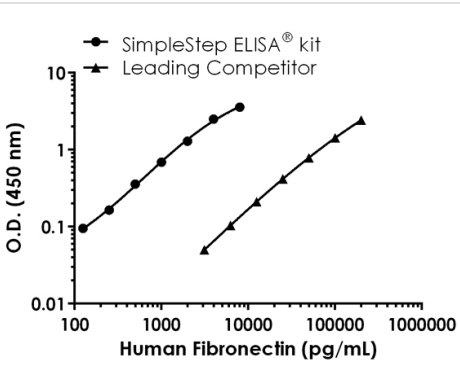
Interpolated concentrations of native Fibronectin in human serum and plasma samples.

The concentrations of Fibronectin were measured in duplicates, interpolated from the Fibronectin standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 1/20,000, plasma (citrate) 1/20,000, and plasma (EDTA) 1/40,000. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibronectin concentration was determined to be 126.5 µg/mL in serum, 141.5 µg/mL in plasma (citrate), and 232.1 µg/mL in plasma (EDTA).



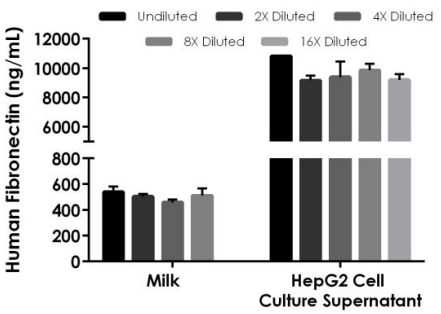
Example of human Fibronectin standard curve in 1X Cell Extraction Buffer PTR.

Background-subtracted data values (mean +/- SD) are graphed.



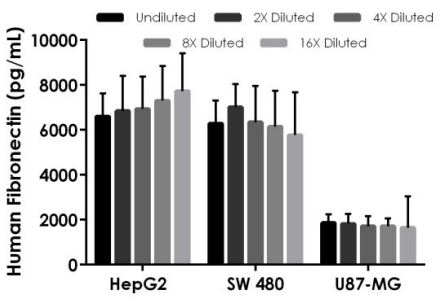
Human Fibronectin standard curve comparison data

Standard curve comparison between human Fibronectin SimpleStep ELISA® kit and traditional ELISA kit from leading competitor. SimpleStep ELISA kit shows comparable sensitivity.



Interpolated concentrations of native Fibronectin in human breast milk and HepG2 cell culture supernatant (4 days) samples.

The concentrations of Fibronectin were measured in duplicates, interpolated from the Fibronectin standard curves and corrected for sample dilution. Undiluted samples are as follows: milk 1/100, and HepG2 cell culture supernatant 1/2000. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibronectin concentration was determined to be 502.9 ng/mL in milk, and 9683 ng/mL in HepG2 cell culture supernatant.

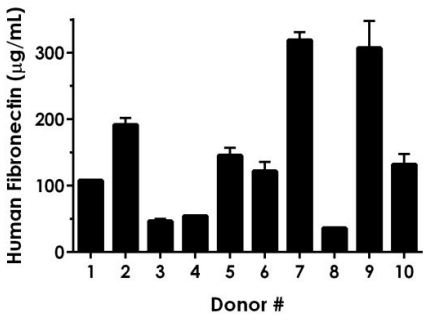


Interpolated concentrations of native Fibronectin in HepG2, SW 480 and U87-MG cell extracts.

Interpolated concentrations of native Fibronectin in human HepG2 cell extract samples based on a 25 µg/mL extract load, SW 480 cell extract samples based on a 100 µg/mL extract load, and U87-MG cell extract samples based on a 5 µg/mL extract load. The concentrations of Fibronectin were measured in duplicate and interpolated from the Fibronectin standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibronectin concentration was determined to be 7085 pg/mL in HepG2 cell extract, 6314 pg/mL in SW 480 cell extract, and 1749 pg/mL in U87-MG cell extract.

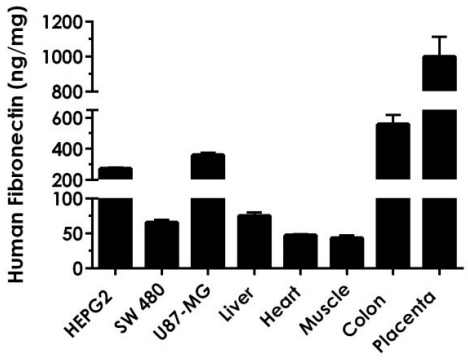
Interpolated concentrations of native Fibronectin in various tissue extracts.

Interpolated concentrations of native Fibronectin in human liver tissue extract based on a 100 µg/mL extract load, heart tissue extract based on a 100 µg/mL extract load, skeletal muscle based on a 100 µg/mL extract load, colon tissue extract based on a 5 µg/mL extract load, and placenta tissue extract based on a 1.25 µg/mL extract load. The concentrations of Fibronectin were measured in duplicate and interpolated from the Fibronectin standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibronectin concentration was determined to be 7634 pg/mL in liver tissue extract, 4910 pg/mL in heart tissue extract, 4278 pg/mL in skeletal muscle tissue extract, 2684 pg/mL in colon tissue extract, and 1224 pg/mL in placenta tissue extract.



Serum from ten individual healthy male human donors was measured in duplicate.

Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibronectin concentration was determined to be 146.2 µg/mL with a range of 35.99 – 319.2 µg/mL.



Interpolated concentrations of native Fibronectin in human cell and tissue extract samples.

The concentrations of Fibronectin were measured in three different dilutions in duplicate and interpolated from the Fibronectin standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted in ng of Fibronectin per mg of extract (mean +/- SD, n=3). Fibronectin concentration was determined to be 271.8 ng/mg in HepG2 cell extract, 65.52 ng/mg in SW 480 cell extract, 359.6 ng/mg in U87-MG cell extract, 75.22 ng/mg in liver tissue extract, 47.20 ng/mg in heart tissue extract, 43.52 ng/mg in skeletal muscle tissue extract, 557.9 ng/mg in colon tissue extract, and 998.8 ng/mg in placenta tissue extract samples.

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