Datasheet for ABIN612798

Fibronectin ELISA Kit

1 Publication

Go to Product page

Overview

Quantity:	96 tests
Target:	Fibronectin
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	3.125-200 ng/mL
Minimum Detection Limit:	3.125 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Mouse Fibronectin ELISA (Enzyme-Linked Immunosorbent Assay) Kit is designed for detection of fibronectin in mouse plasma, serum, urine, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures mouse fibronectin in approximately 4 hours. A polyclonal antibody specific for mouse fibronectin has been pre-coated onto a 96-well microplate with removable strips. Fibronectin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for mouse fibronectin, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
Brand:	AssayMax™
Sample Type:	Cell Culture Cells, Plasma, Serum, Urine
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Mouse Fibronectin Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against mouse fibronectin. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Mouse Fibronectin Standard: Mouse fibronectin in a buffered protein base (300 ng, lyophilized). Biotinylated Mouse Fibronectin Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against mouse fibronectin (120 l). MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles). SP Conjugate (100x): A 100-fold concentrate (80 l). Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution (1x): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction (12 ml).
Material not included:	Microplate reader capable of measuring absorbance at 405 nm. Pipettes (1-20 µL, 20-200 µL, and multiple channel). Deionized or distilled reagent grade water. Incubator (37 °C)

Target Details

Target:	Fibronectin
Alternative Name:	Fibronectin (FN) (Fibronectin Products)
Background:	Fibronectin (FN) is a major component of the extracellular matrix and blood plasma. It is also a specific ligand for several integrin adhesion receptors (1). FN plays an important role not only in cell adhesion (2) and wound healing (3), but also in embryogenesis (4) and hematopoiesis (5).
Gene ID:	14268
UniProt:	P11276

Application Details

Sample Volume:	50 µL
Assay Time:	4 h
Plate:	Pre-coated
Protocol:	• Step 1. Add 50 µL of Standard or Sample per well. Incubate 2 hours. • Step 2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour. • Step 3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes. • Step 4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 10 minutes.

	• Step 5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8 °C. Mouse Fibronectin Standard: Reconstitute the Mouse Fibronectin Standard (300 ng) with 1.5 mL of MIX Diluent to generate a 200 ng/mL standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (200 ng/mL) 2-fold with equal volume of MIX Diluent to produce 100, 50, 25, 12.5, 6.25, and 3.125 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining stock solution should be stored at -20 °C and used within 30 days. Avoid repeated freeze-thaw cycles. Standard Point Dilution [Mouse FN] (ng/mL) P1 1 part Standard (200 ng/mL) 200 P2 1 part P1 + 1 part MIX Diluent 100 P3 1 part P2 + 1 part MIX Diluent 50 P4 1 part P3 + 1 part MIX Diluent 25 P5 1 part P4 + 1 part MIX Diluent 12.5 P6 1 part P5 + 1 part MIX Diluent 6.25 P7 1 part P6 + 1 part MIX Diluent 3.125 P8 MIX Diluent 0.0 Biotinylated Mouse Fibronectin Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent to produce a 1x solution. The undiluted antibody should be stored at -20 °C. Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20 °C. 5
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. An 8000-fold sample dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. An 8000-fold sample dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. The sample is suggested for use at 1x, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell

	culture media at 3000 x g for 10 minutes at 4 °C to remove debris and collect supernatants. Samples can be stored at -20 °C or below. Avoid repeated freeze-thaw cycles.
Assay Procedure:	Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 l of Mouse Fibronectin Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition. Wash five times with 200 l of Wash Buffer manually. Invert the plate each time and decant the contents, hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 l of Wash Buffer and then invert the plate, decanting the contents, hit 4-5 times on absorbent material to completely remove the liquid. Add 50 l of Biotinylated Mouse Fibronectin Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour. Wash the microplate as described above. Add 50 l of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. Add 50 l of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 10 minutes or until the optimal blue color density develops. Add 50 l of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings. 6
Calculation of Results:	• Calculate the mean value of the duplicate or triplicate readings for each standard and sample. • To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. • Determine the unknown sample concentration from the standard curve and multiply the value by the dilution factor.

Application Details

Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.8% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	This product is for Research Use Only and is not intended for use in diagnostic procedures. Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor. Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents. The Stop Solution is an acidic solution. The kit should not be used beyond the expiration date. 2
Storage:	4 °C/-20 °C
Storage Comment:	Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date. Store SP Conjugate and Biotinylated Antibody at -20°C. Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator. Diluent (1x) may be stored for up to 30 days at 2-8°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Publications

Product cited in:	Wang, Hirase, Nitto, Soma, Node: "Eicosapentaenoic acid increases cytochrome P-450 2J2 gene expression and epoxyeicosatrienoic acid production via peroxisome proliferator-activated receptor γ in endothelial cells." in: **Journal of cardiology**, Vol. 54, Issue 3, pp. 368-74, (2009) (PubMed). Larsen, Miura, Hatoum, Campbell, Hammock, Zeldin, Falck, Gutterman: "Epoxyeicosatrienoic and dihydroxyeicosatrienoic acids dilate human coronary arterioles via BK(Ca) channels: implications for soluble epoxide hydrolase inhibition." in: **American journal of physiology. Heart and circulatory physiology**, Vol. 290, Issue 2, pp. H491-9, (2006) (PubMed). Oltman, Weintraub, VanRollins, Dellsperger: "Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are potent vasodilators in the canine coronary microcirculation." in: **Circulation research**, Vol. 83, Issue 9, pp. 932-9, (1998) (PubMed).

Fang, Kaduce, Weintraub, VanRollins, Spector: "Functional implications of a newly characterized pathway of 11,12-epoxyeicosatrienoic acid metabolism in arterial smooth muscle." in: **Circulation research**, Vol. 79, Issue 4, pp. 784-93, (1996) (PubMed).