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Product Manual

Fascin ELISA Kit

Catalog Number : 82001

96 assays

NewEast Biosciences
24 Whitewoods Lane
Malvern, PA 19355

Fax: 610-945-2008
Phone: 610-945-2007
Web: www.neweastbio.com
E-mail: sale@neweastbio.com

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FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES

Product Description

Fascin is an ~58 kDa monomeric actin filament bundling protein. It is required to maximally cross-link the actin filaments into straight, compact, and rigid bundles, and contributes to the formation of filopodia that are critical for cell migration. Elevated levels of fascin have been found in metastatic tumors and are correlated with clinically aggressive phenotypes, poor prognosis, and shorter survival.

NewEast Biosciences fascin ELISA Kit is a simple and fast sandwich enzyme immunoassay (EIA) for in vitro quantitative detection of fascin proteins in cell lysates, blood and urine samples. Each kit provides sufficient quantities to perform 96 assays. It works for fascin proteins for all vertebrates.

Assay Principle

With the NewEast Biosciences fascin sandwich ELISA assay system, rabbit polyclonal antibodies generated against fascin proteins, are pre-coated onto a 96-well plate and are used to capture fascin proteins from a sample. Captured fascin is detected using fascin specific mouse monoclonal antibodies and a goat anti-mouse horse radish peroxidase conjugate. After addition of the substrate solution, the amount of fascin is determined. The standard curve demonstrates a direct relationship between Optical Density (OD) and fascin concentration: i.e., the higher the OD the higher the fascin concentration in the sample.

Kit Components

1. Immunoplate Pre-coated with Rabbit anti-fascin Polyclonal Antibody (Catalog No.30401): One Plate of 96 Wells. A plate using break-apart strips coated with Rabbit anti-fascin Polyclonal Antibody.

2. Assay/Diluent Buffer (Catalog No. 30403): One bottle 30 mL of 100 mM phosphate buffer, pH 7.2, 1% BSA, 0.1% Tween-20 and 0.02% Thimerosol.

3. Anti-fascin Mouse Monoclonal Antibody (Catalog No. 26010): One vial 10 µL (200 µg/ml) in PBS, pH 7.4 (a 2000 X stock solution and diluent buffer are provided).

4. Goat anti-Mouse horse radish peroxidase conjugated antibody (Catalog No. 29001): One vial 10 µl (200 µg/ml) in PBS, pH 7.4, containing 50% glycerol(a 3000 X stock solution and diluent buffer are provided).

5. 10X Wash Buffer Concentrate (Catalog No. 30106): One bottle 15 mL phosphate buffered saline containing detergents.

6. Fascin Standard (Catalog No. 30402): One vial (15 µg in 150 µl) of human fascin protein standard.

7. Substrate A (Catalog No. 30107): One vial 6 mL.

8. Substrate B (Catalog No. 30108): One vial 6 mL.

9. Stop Solution (Catalog No. 30110): One vial 12 mL.
   A solution of oxalic acid in water. Keep tightly capped.

Storage

Store all kit components at 4°C until their expiration dates.
Note: For long-term best results, store anti-fascin Mouse Monoclonal Antibody (Catalog No. 26010), fascin standard (Catalog No. 30402) at -80℃upon receipt. Add 1 μL anti-fascin mouse monoclonal antibody into 2 mL diluent buffer and mix the solution gently before use. Please avoid repeated thawing and freezing after mixing.

Materials Needed but Not Supplied

1. Multi-channel or repeating pipettes
2. Pipettors & tips capable of accurately measuring 10-1000 μL
3. Graduated serological pipettes
4. 96-well microplate reader capable of measuring absorbance at 405 nm
5. Graph paper for manual plotting of data
6. 1.5 mL tubes
7. Mechanical vortex
8. Two1 liter containers
9. Plate shaker (optional)
10. Distilled or deionized water

Reagent Preparation

1. Fascin Standard

Allow the fascin standard solution to warm to room temperature. Label nine (or more) tubes #1 through #9. Pipet 540 μL assay diluent into tube #1 and 300 μL assay diluent into tubes #2-9. Add 60 μL of the 1.82 μmol/L fascin standard to tube #1. Vortex thoroughly. Add 300 μL of tube #1 to tube #2 and vortex thoroughly. Continue this for tubes #3 through #6.

The concentration of fascin in tubes #1 through #9 will be 182, 91, 45.5, 22.75, 11.4, 5.69, 2.8, 1.4 and 0.7 nmol/L, respectively.
Diluted standards should be used within 30 minutes of preparation.

2. Anti-fascin mouse monoclonal antibody
Immediately before use, dilute the anti-fascin mouse monoclonal antibody 1:2000 with assay/diluent buffer as follows: For each 20 well strip, prepare 2 mL of diluted anti-fascin antibody by adding 2 µL of anti-fascin mouse monoclonal antibody to 1998 µL of assay/diluent buffer.

3. Goat anti-Mouse Polyclonal Antibody, horse radish peroxidase conjugated
Immediately before use, dilute the goat anti-mouse horse radish peroxidase conjugate 1:3,000 with ELISA Assay/Diluent buffer as follows: For each 30 well strip prepare 3 mL of diluted goat anti-mouse horse radish peroxidase conjugate by adding 1 µL of goat anti-mouse horse radish peroxidase conjugate antibody to 2999 µL of Assay/Diluent buffer.

4. Wash Buffer
Prepare the Wash Buffer by diluting 15 mL of the supplied concentrate with 135 mL of deionized water. This can be stored at room temperature until the kit expiration date.

Sample Preparation

Adherent Cells

1. Culture cells (one 10-cm plate, ~ 10^7 cells) to approximately 80-90% confluence. Stimulate cells with activator or inhibitor as desired.
2. Aspirate the culture media and wash twice with ice-cold PBS.
3. Completely remove the final PBS wash and add ice-cold 1X Assay/Lysis Buffer to the cells (0.5 - 1 mL per 10 cm tissue culture plate).
4. Place the culture plates on ice for 10-20 minutes.
5. Detach the cells from the plates by scraping with a cell scraper.
6. Transfer the lysates to appropriate size tubes and place on ice.
7. If nuclear lysis occurs, the cell lysates may become very viscous and difficult to pipette. If this occurs, lysates can be passed through a 27½-gauge syringe needle 3-4 times to shear the genomic DNA.
8. Clear the lysates by centrifugation for 10 minutes (12,000 x g at 4 °C).
9. Collect the supernatant and store samples (~1-2 mg of total proteins) on ice for immediate use,

or snap freeze and store at - 70 °C for future use.

Suspension Cells

1. Culture cells and stimulate with activator or inhibitor as desired.
2. Perform a cell count, and then pellet the cells by centrifugation.
3. Aspirate the culture media and wash twice with ice-cold PBS.
4. Completely remove the final PBS wash and add ice-cold 1X Assay/Lysis Buffer to the cell pellet (0.5 – 1 mL per 1 x 10^7cells).
5. Lyse the cells by repeated pipetting.
6. Transfer the lysates to appropriate size tubes and place on ice.
7. If nuclear lysis occurs, the cell lysates may become very viscous and difficult to pipette. If this occurs, lysates can be passed through a 27½-gauge syringe needle 3-4 times to shear the genomic DNA.
8. Clear the lysates by centrifugation for 10 minutes (12,000 x g at 4 °C).
9. Collect the supernatant and store samples on ice for immediate use, or snap freeze and store at -70 °C for future use.

Blood Samples

1. Blood can be assayed directly, but for most assay purposes, it is either allowed to clot or is centrifuged down to pellet out red and white cells.

2. Clotting requires several hours at room temperature, and the clear yellow liquid, the serum, can be used for fascin analysis. More rapid and convenient is to spin the blood down at top speed in an Eppendorf centrifuge and take the clear yellow liquid, the plasma, for analysis. Workable results have been obtained with blood, serum and plasma, but the kit has been standardized on plasma for reproducibility.

3. A series of blood samples can be taken and frozen at -20°C or lower. Then, when a complete series of samples have been collected they can be thawed out and the red and white cells can be pelleted out in microfuge tubes at top speed for 5 minutes in a microfuge centrifuge. The plasma is then run in the ELISA.

Assay Procedure

1. Place the desired number of strips in the strip well plate holder. Return unused strips to the foil pouch. Tape may assist in holding the wells in place during the assay.

2. Add 100 µL of standards or samples to wells (see reagent preparation section). It is recommended that standards and samples be run in duplicate.

Note: A standard curve must be run at each setting.

3. Seal the plate with a plate sealer. Incubate the plate for 1 hour at 37℃ with gentle shaking.

4. **IMPORTANT WASH STEP:**
Gently remove the plate sealer and wash the plate at least 3 times. A thorough washing of the plate is extremely important to reduce background. We recommend using a multi-channel pipette to fill each well with 200 µL of diluted Wash Buffer. Fluid removal from the wells is best accomplished by inverting the plate over a sink and flicking the fluid out of the wells and then blotting the plate on clean paper towels. Repeat this procedure for a total of 3 times.

5. Add 100 µL of the diluted anti-fascin mouse monoclonal antibody (see reagent preparation section) to each well. Cover the plate and incubate the plate for 1 hour at 37℃ with gentle shaking.

6. Wash as described in Step 4.

7. Add 100 µL of the diluted horse radish peroxidase conjugated goat anti-mouse polyclonal antibody (see reagent preparation section) to each well. Cover the plate and incubate the plate for 1 hour at 37℃ with gentle shaking.

8. Wash as described in Step 4.

9. Add 100 µL of the Substrate solution to every well. Incubate at room temperature for 5~30 minutes without shaking. (**Substrate A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light.)**

10. Add 100 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.

11. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

CAUTION: *Bubbles in the wells will cause inaccurate readings. Ensure that all bubbles are removed prior to taking the absorbance reading.*

Calculation of Results

Manual Plotting

Plot the standard curve on graph paper. Known concentrations of fascin are plotted on the X-axis and the corresponding OD on the Y-axis. The standard curve should result in a graph that shows a direct relationship between fascin concentrations and the corresponding ODs. In other words, the greater the concentration of fascin in the sample, the higher the OD. The concentration of fascin in unknown samples may be determined by plotting the sample OD on the Y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line dropped from this point intersects the X-axis at the concentration of fascin in the unknown sample.

Plate Reader/PC Interface

An alternative approach is to enter the data into a computer program curve fitting software. A good fit can be obtained with a linear regression analysis. Some data points at the top or bottom of the range tested may need to be dropped to get a good fit. Currently existing spreadsheet software can perform such plotting.

Typical Standard Curves

These curves **must not** be used to calculate fascin concentrations; each user must run a standard curve for each assay and version used.