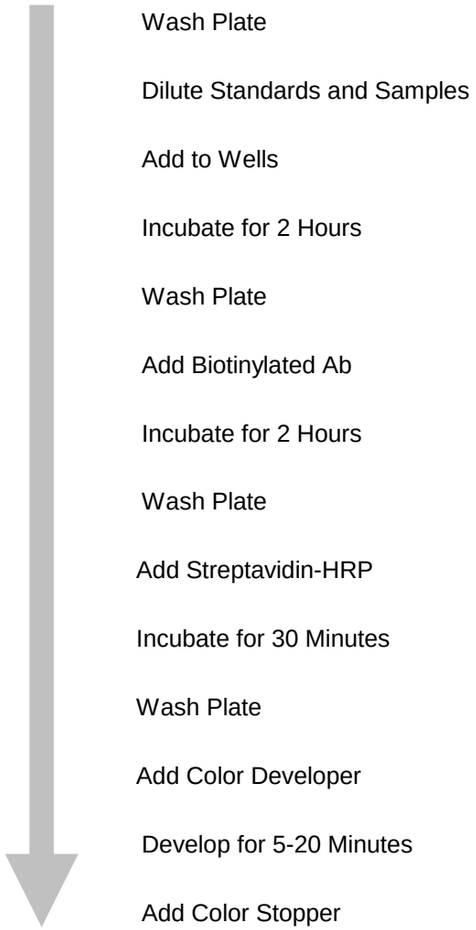
βIG-H3-M ELISA



FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC PROCEDURES

βIG-H3-M ELISA: An ELISA designed to measure βig-h3 in urine, serum or cell culture supernatant of mouse origin.

Intended Use: βIG-H3-M ELISA is a quantitative sandwich ELISA for measurement of mouse βig-h3 found in urine, serum, and supernatant. It is for research purposes, and is not intended for diagnostic use.

Technical Background: βIG-H3 (beta-induced gene h3) is a 68 kD protein induced in diverse cell types by Transforming Growth Factor-ß (TGF-ß) that is secreted into the extra-cellular matrix and is involved in cell growth, differentiation, adhesion, and wound healing. ßIG-H3 expression is altered in various conditions including tumorigenesis, breast cancer, corneal dystrophy, osteogenesis, and atherothrombosis. ßig-h3 is elevated in the urine of patients with cyclosporine nephrotoxicity and with type 2 diabetes in whom it shows positive correlation with the albumin excretion rate, making it a useful biomarker of renal dysfunction due to diabetes in its earliest phases and in monitoring clinical progression of diabetic nephropathy.

βIG-H3-M ELISA uses a 96-well assay plate pre-coated with sheep anti-mouse βIG-H3 as capture antibody. Appropriately diluted recombinant mouse βIG-H3 standard and samples bind to the capture antibody. The βIG-H3 is then labeled with a biotinylated polyclonal sheep anti-mouse βIG-H3 antibody. A subsequent reaction with Streptavidin-HRP conjugate labels the biotinylated probe with enzyme. After washing, only the βIG-H3 bound to the capture antibody on the stationary phase will be detected using a chromogenic reaction. Color intensity in βIG-H3-M ELISA is proportional to the amount of βIG-H3 captured on the plate.

Specimen Collection and Storage: Collect samples without preservative, separate and clarify them by centrifugation if necessary. Samples may be stored at 4˚C for up to 1 week or at -60˚C for up to 2 months. Prior to assay, allow the samples to come to room temperature. Do not apply heat to thaw frozen samples.

Kit Contents: The βIG-H3-M ELISA kit contains the following items:

a. 1 βIG-H3-M Assay Plate
b. 1 βIG-H3-M Diluent
c. 1 βIG-H3-M Standard
d. 1 Biotinylated sheep anti-βIG-H3-M
e. 1 Streptavidin-HRP Conjugate
f. 1 Color Developer
g. 1 Color Stopper
h. 1 Instructions

Specimen Required: Urine, serum or tissue extract. (Mouse)

*Assay Range:*0.156 – 10.0 ng/ml
Precision: Intra- and inter-assay precision for samples within the assay range have a C.V. of <10%.

Materials Needed:

1. Wash Buffer-0.05% Tween20 in PBS, pH 7.2-7.4
2. ELISA plate reader capable of reading at 450 nm with reference at 630 nm
3. Microfuge tubes for dilution of standard and samples.

Assay Procedure:

1. Bring all kit reagents to room temperature.
2. Serial dilutions of βIG-H3-M Standard (2x concentrate) are made by adding 225 µl of βIG-H3-M Diluent to 6 tubes numbered 2 through 7. Starting with standard vial containing 2x concentrate, remove 225 µl and add to tube #2 and mix, remove 225 µl and add to tube #3 and mix. Continue with tubes #4 through #7. This will constitute a standard curve.
3. Dilute samples to the appropriate concentration with βIG-H3-M Diluent, but at least 1:2 (undiluted samples should be avoided). A dilution series for unknowns of 1:5, 1:10 and 1:20 should be appropriate for most diabetic samples.
4. Aspirate each well and wash with wash buffer a total of three times

by filling each well with wash buffer and removing the liquid, after the last wash remove any remaining wash buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

5. Add 100 µl of βIG-H3-M Diluent to wells A1 and A2, this will be your negative control or blank wells. Add 100 µl of standard from stock vial to wells B1 and B2, this is standard #1 containing 10.0 ng/well; add 100 µl from tube #2 to wells C1 and C2; add 100 µl from tube #3 to wells D1 and D2; add 100 µl from tube #4 to wells E1 and E2; add 100 µl from tube #5 to wells F1 and F2; add 100 µl from tube #6 to wells G1 and G2; and add 100 µl from tube #7 to wells H1 and H2.
6. Add 100 µl Sample to appropriate wells and record position on a plate map.
7. Incubate for 2 hours at room temperature in a moist chamber.
8. Wash the plate 3 times with Wash Buffer as in step 4.
9. Add 100 µl of Biotinylated sheep anti-βIG-H3-M.
10. Incubate for 2 hours at room temperature in a moist chamber.
11. Wash the plate 3 times with Wash Buffer as in step 4.
12. Add 100 µl of Streptavidin-HRP Conjugate to each well.
13. Incubate for 30 minutes at room temperature in a moist chamber.
14. Wash the plate 3 times with Wash Buffer as in step 4.
15. Add 100 µl of Color Developer to each well.
16. Allow blue color to develop for 5-20 minutes.
17. Add 100 µl of Stop Solution to each well.
18. Read in ELISA plate reader at 450 nm. Reference blank is at 630 nm.

Calculation of Results:

Average the duplicate readings for each standard and sample and subtract the average zero (blank) optical density. Create a standard curve using computer software capable of a four parameter logistic curve fit. As an alternative, construct a standard curve by plotting the absorbance against the concentration of βig-h3 and draw a best fit curve. The data may be linearized by plotting the log absorbance against the log concentration and the best fit line can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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Trouble Shooting:

1. No color appears after adding Color Developer: One or more reagents may have been adversly affected by storage above 8ºC. One or more reagents may not have been added. Repeat assay. Be sure to store the kit appropriately.
2. Color in wells too light: Longer incubation with Color Developer may be required. If the color is still too light after 10 minutes development, repeat the assay but increase the primary and secondary incubations to 4 hours each.
3. Color in wells is too dark: Decrease the development time. If a 5 minute development is still too dark, repeat the assay and reduce the primary and secondary incubations to 1 hour each. If color is dark and the standard dilutions fail to show the appropriate dose-response, Color Developer may have been contaiminated with conjugate or the plate was poorly washed. Repeat the assay and take care in the pipetting and in the washing operations.
4. Color in sample well(s) is darker or lighter than lowest or highest concentrations of the standard curve. Change sample dilution protocol appropriately.
5. Poor agreement between duplicate wells: This is almost always due to pipetting error. Repeat the assay. Microplate ELISAs may be prone to edge effects wherein the outer rows and columns show a darker response than the inner ones. This effect may be minimized by incubating the plate in a closed humid container. A plastic food storage container with a tight fitting lid and a water moistened paper towel work well in this respect. Place the moistened towel in the bottom of the container, and place the plate upon it. Position the cover and incubate as described.

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