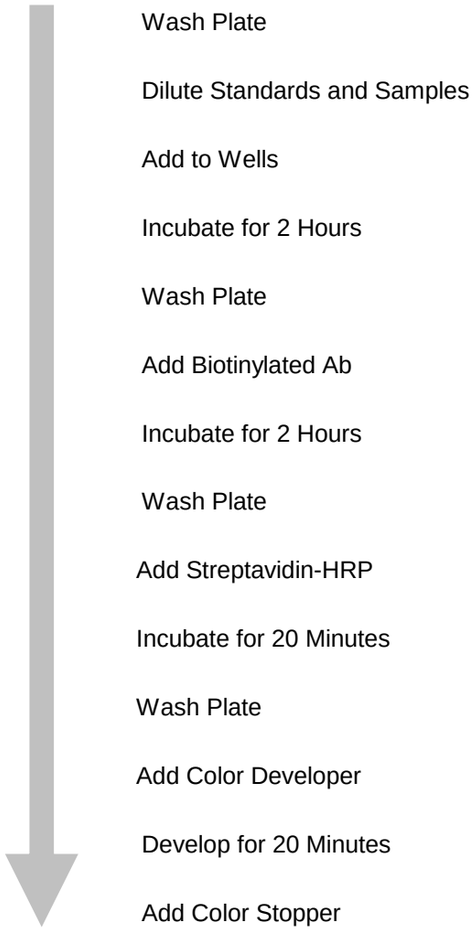
TNF-sR2 ELISA



FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC PROCEDURES

TNF-sR2 ELISA: An ELISA designed to measure Tumor Necrosis Factor Soluble Receptor II of human origin in plasma, serum, urine or cell culture supernatant.

Intended Use: TNF-sR2 ELISA is a quantitative sandwich ELISA for measurement of humanTNF-sRII found in plasma, serum, and supernatant. It is for research purposes, and is not intended for diagnostic use.

Technical Background: TNF-sR2 ELISA measures the soluble receptor II of TNF *α* in urine, plasma, serum or cell culture supernatant of human origin. The 55-kD TNFαR1 and 75-kD TNF αR2 are specific cell surface receptors for TNF α, an inflammatory-mediating cytokine, that elicits TNF-mediated responses through activation of cell signaling pathways. TNF α and its receptors are shed from the cell surface, with TNF α appearing in the plasma as free cytokine or bound to circulating receptors released into the extracellular space. Circulating concentrations of TNF αRII have been found to be associated with cardiovascular and renal disease, and particularly with the development of chronic kidney disease in diabetes.

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 TNF-sR2 ELISA kit contains the following items:

a. 1 TNF-sR2 Assay Plate
b. 2 TNF-sR2 Diluent
c. 1 TNF-sR2 Standard
d. 1 Biotinylated goat anti-TNF-sR2
e. 1 TNF sR2 Diluent with NGS
f. 1 Streptavidin-HRP Conjugate
g. 1 Color Developer
h. 1 Color Stopper
i. 1 Instructions

Specimen Required: Tissue culture supernatant, urine, serum or plasma (human).
*Assay Range:*15.6 to 1000 pg/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. The first standard is 100ul taken directly out of the TNF-sR2 standard vial and added to the plate.Serial dilutions of TNF-sR2 Standard are made by adding 250ul of TNF-sR2 Diluent to 6 tubes numbered 2 through 7. Starting with standard vial containing 1000 pg/ml remove 250 µl and add to tube #2 and mix, remove 250 µl and add to tube #3 and mix. Continue with tubes #4 through #7. This will constitute a standard curve ranging from 15.6 to 1000 pg/ml.
3. Dilute samples to the appropriate concentration with TNF-sR2 Diluent A dilution series for plasma or serum of 1:2, 1:5, 1:10 should be appropriate for most diabetic samples. Initial dilutions can be made in PBS then the final dilution added to the test can be made in Reagent Diluent.
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 TNF-sR2 Diluent to wells A1 and A2, this will be your negative control or blank wells. Add 100 µl of standard from theTNF-sR2 Standard vial to wells B1 and B2, this is standard #1 containing 1000 pg/ml; 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. The Biotinylated goat anti-TNF-sR2 stock(100ul) must be diluted into the 12 ml TNF-sR2 Diluent with NGS (12ml) before use. Then add 100 ul to each well.
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 20 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 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 TNF-sR2 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 20 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.

Disclaimer: Exocell makes no guarantee of any kind, expressed or implied, which extends beyond the description of the material in this kit, except that these materials and this kit will meet our specifications at the time of delivery. Customer's remedy and Exocell's sole liability hereunder is limited, at Exocell's option, to replacement to material that does not meet our specifications. By acceptance of our products, the customer indemnifies and holds Exocell harmless against, and assumes all liability for, the consequences of its use or misuse by the customer, its employees or others.

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