Human Tissue Factor ELISA Kit
Cat#: orb96809 (ELISA Manual)

Assay Principle

Itis a solid phase immunoassay specially designed to measure Human F3 with a 96-well strip plate that is pre-coated with antibody specific for F3. The detection antibody is a biotinylated antibody specific for F3. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human F3 with immunogen: Expression system for standard: NSO; Immunogen sequence: S33-E251. The kit is analytically validated with ready to use reagents.

To measure Human F3, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly propotional to Human F3 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human F3 in the sample. For more information on assay principle, protocols, and troubleshooting tips,

Overview

Product Name	Human Tissue Factor/F3 PicoKine™ ELISA Kit
Reactive Species	Human
Size	96wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human Tissue factor/F3. 96wells/kit, with removable strips.
Sensitivity	<10pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	15.6pg/ml-1000pg/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles(Shipped with wet ice.)
Uniprot ID	P13726

Technical Details

Capture/Detection Antibodies	The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.
Specificity	Natural and recombinant Human F3
Immunogen	Expression system for standard: NSO; Immunogen sequence: S33-E251
Cross Reactivity	There is no detectable cross-reactivity with other relevant proteins.

Kit Components/Materials Provided

Description	Quantity	Volume
Anti-Human F3 Pre-coated 96-well strip microplate	1	12 strips of 8 wells
Human F3 Standard	2	10ng/tube
Human F3 Biotinylated antibody (100x)	1	130 µl
Avidin-Biotin-Peroxidase Complex (100x)	1	130 µl
Sample Diluent	1	30ml
Antibody Diluent	1	12ml
Avidin-Biotin-Peroxidase Diluent	1	12ml
Color Developing Reagent (TMB)	1	10ml
Stop Solution	1	10ml
Plate Sealers	4	Piece

Required Materials That Are Not Supplied

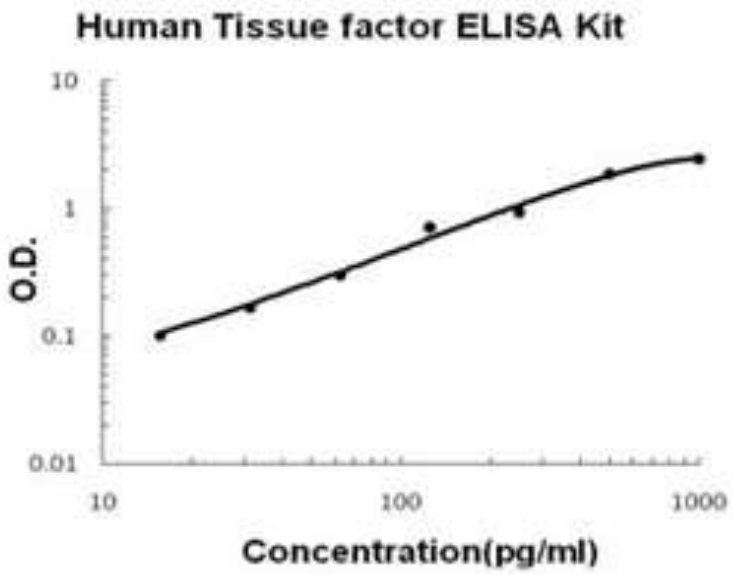
- Microplate Reader capable of reading absorbance at 450nm.
- Automated plate washer (optional)
- Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
- Multichannel pipettes are recommended for large amount of samples.
- Deionized or distilled water.
- 500ml graduated cylinders.
- Test tubes for dilution

Human Tissue Factor/F3 PicoKine™ ELISA Kit (EK0928) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration(pg/ml)	0	15.6	31.2	62.5	125	250	500	1000
O.D	0.031	0.101	0.165	0.296	0.708	0.934	1.851	2.431

Human Tissue factor/F3 PicoKine ELISA Kit standard curve



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Intra/Inter Assay Variability Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess inter-assay
precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	28	204	455	28	205	493
Standard deviation	1.26	13.46	34.58	1.54	14.14	40.91
CV(%)	4.5%	6.6%	7.6%	5.5%	6.9%	8.3%

Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	28	29	31	27	28	1.47	5.2%
Sample 2	204	231	201	203	209	12.31	5.8%
Sample 3	455	425	389	431	425	23.62	5.5%

*number of samples for each test n=20.

Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-20min) is based on 37°C.
Wash buffer	Dissolve the included powder in 1000ml of deionized water. Excess wash buffer can be stored for up to one week at 4°C.
Biotinylated Anti-Human F3 antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human F3 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human F3 Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10ng of lyophilized Human F3 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Dilution of Human F3 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –1000pg/ml, #2 –500pg/ml, #3 – 250pg/ml, #4 – 125pg/ml, #5 – 62.5pg/ml, #6 – 31.25pg/ml, #7 – 15.625pg/ml, #8 – 0.0 (Blank).

2. To generate standard #1, add 100µl of the reconstituted standard stock solution of 10ng/ml and 900µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.

3. Add 300 µl of sample diluent to tubes # 2-7.

4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.

5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.

6. Continue the serial dilution for tube #4-7.

7. Tube #8 is a blank standard to be used with every experiment.

Sample Preparation and Storage

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
Plasma	Collect plasma using heparin, EDTA or citrate as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.
Urine	Collect the first urine of the day, micturate directly into a sterile container. Remove impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.
Cell lysates	Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10000 X g for 5 min. Collect the supernatant.

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. At least two replicates of each standard. sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Human F3 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
    b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
    c. Repeat steps a-b 2 additional times.

1. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well and incubate for 40 minutes at RT (or 30 minutes at 37°C).
2. Wash the plate 5 times with the 1x wash buffer.
    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
    b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
    c. Repeat steps a-b 4 additional times.
3. Add 90 µl of Color Developing Reagent to each well and incubate in the dark for 30 minutes at RT (or 25-30 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
4. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
5. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. A free program capable of generating a four-parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.