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SKU IP800023

ELISA KIT FOR HUMAN FA

Research Use Only
For *in vitro* applications - not for consumption

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VERSION 1.0

INTENDED USE

The kit is a competitive inhibition enzyme immunoassay for in vitro quantitative measurement of FA (Folic Acid) in human serum, plasma, tissue homogenates, cell lysates, urine, saliva, cell culture supernates and other biological fluids.

KIT CONTENTS & STORAGE

Precoated Microplate [12 X 8 wells]
Standard (lyophilized) [2 vials]
Standard Diluent Buffer [20mL]
Biotinylated-Conjugate (100×) [60μL]
Biotinylated Conjugate Diluent [10mL]
Streptavidin-HRP (100×) [120μL]
HRP Diluent [12mL]
Wash Buffer (25×) [20mL]
TMB Substrate Solution [9mL]
Stop Reagent [6mL]
Plate Sealing Membrane [2 pcs]
User manual [1pc]

Note: *Store all the components at 4°C.*

MATERIALS REQUIRED BUT NOT SUPPLIED

ELISA Reader, 37°C Thermostat, micropipettes and tips, Eppendorf tubes, absorbent paper, autowasher, deionized or distilled water.

SUMMARY

The microtiter plate wells provided in this kit has been pre-coated with an antibody specific to FA. Standards or samples are added to the appropriate microtiter plate wells then incubated with a biotin-conjugated antibody specific to FA. Next, Avidin conjugated to HRP is added to each microplate well and incubated. After that, TMB substrate solution is added. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of FA in the samples is then determined by comparing the OD of the samples to the standard curve.

CHARACTERISTICS

Sample Type: Serum, plasma, tissue homogenates, cell lysates, urine, saliva, cell culture supernates and other biological fluids.

Detection Range: 156.25-10000 pg/mL
Minimum Detection Dose: 14.7 pg/mL
Intra-Assay Precision: CV<8%
Inter Assay Precision: CV<10%
Linearity with spiked analytes: ρ=0.99
Cross Reactivity: None observed with analogues
Validity: 1 year from the date of manufacturing
Storage: 2-8°C

Once opened, unused wells should be put into the sealed bag with a desiccant pack and stored at 2-8 °C.

SAMPLE COLLECTION

Tissue homogenates - Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces and homogenized in PBS with a glass homogenizer on ice. Different lysis buffer needs to be chosen based on subcellular location of the target protein. 1mL lysis buffer should be added in 9 mg tissue sample. Protease inhibitor is recommended to be added into the PBS. To further break the cells, sonication of the suspension may be done with an ultrasonic cell disrupter or they may be subjected to freeze-thaw cycles. The homogenates are centrifugated for 5 minutes at 10,000×g to collect supernatant.

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Cell Lysates - Cells need to be lysed before assaying according to the following directions. 1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly). 2. Wash cells three times in cold PBS. 3. Resuspend cells in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified. 4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤-20°C. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C. Remove particulates and assay immediately or store samples in aliquot at ≤-20°C. Avoid repeated freeze/thaw cycles.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Collect the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

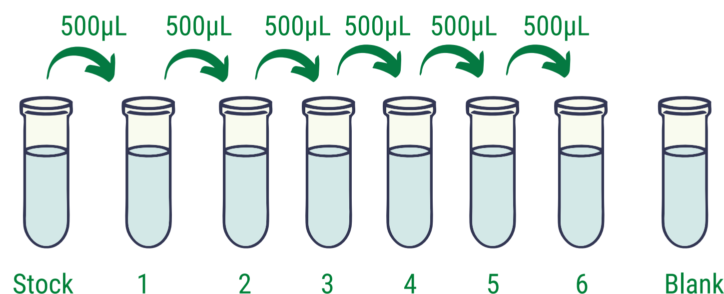
Note:

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be detected.
3. When performing the assay, bring samples to room temperature.
4. Samples should be centrifuged adequately and no hemolysis or granule is to be allowed.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature (18-25°C) before use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.
Predict the concentrations before assaying. If values for these are not within the range of the standard curve, user must determine the optimal sample dilutions for their particular experiment.

2. **Standard** - Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 10000 pg/mL. Please prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 10000 pg/mL, 5000 pg/mL, 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 312.5 pg/mL, 156.25 pg/mL, and the last EP tubes with Standard Diluent is the blank as 0 pg/mL. In order to guarantee the experimental results validity, please use the new standard solution for each experiment.



3. **Biotinylated-Conjugate and Streptavidin-HRP** - Briefly spin or centrifuge the stock Biotinylated Antibody and Streptavidin-HRP before use. Dilute them to the working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively.

4. **Wash Buffer** - Dilute the 25x wash buffer into 1x working concentration with double distilled water.

5. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

PROCEDURE

1. Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

2. Prepare all reagents, working standards, and samples as directed in the previous sections.

3. Set a Blank well with Standard Diluent Buffer. Add 50 µL of Standard or Sample to per well. Add 50µL of Biotinylated -Conjugate(1x) to each well. Mix well, Cover with the adhesive films provided. Incubate for 1 hour at 37°C.

4. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (200μL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100μL of Streptavidin-HRP (1x) to each well. Cover with the adhesive films provided. Incubate for 1 hour at 37°C.

6. Repeat the aspiration, wash process for total 5 times as conducted in step 4.

7. Add 90μL of Substrate Solution to each well. Incubate for 20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Avoid placing the plate in direct light.

8. Add 50μL of Stop Solution to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between human FA concentration in the sample and the assay signal intensity.
Average the duplicate readings for each standard, control, and samples. Create a standard curve with the human FA concentration on the y-axis and absorbance on the x-axis. Using some plot software, for instance, curve expert draw the best fit straight line through the standard points and it 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.

TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

We have compiled a list of most common errors and resolutions on our technical support webpage www.immunoport.com/scientific-support. Still if you have any concerns please feel free to write to us at info@immunoport.com.

PRECAUTIONS

- Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.
- Making serial dilution in the wells directly is not permitted.
- All kit components have been formulated and quality control tested to function successfully as a kit. Do not mix or substitute reagents or materials from other kit, performance cannot be guaranteed if utilized separately or substituted.
- Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly.
- Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally leading to wrong results. Also, bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
- If crystals have formed in the Wash Solution concentrate (25×), warm to room temperature and mix gently until the crystals are completely dissolved.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB is light sensitive. Avoid prolonged exposure to light. Avoid contact of the TMB with metal to prevent color development. TMB is toxic, avoid direct contact with hands. Dispose of properly. If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded.
- Bacterialorfungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.

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