KAMIYA BIOMEDICAL COMPANY

Rat Coagulation Factor X (F10) ELISA

For the quantitative determination of rat F10 in serum, plasma, cell culture fluid and other biological fluids

Cat. No. KT-51154

For Research Use Only. Not for use in diagnostic procedures.

Product Information

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INTENDED USE

This F10 ELISA kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures. The stop solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of F10 in the sample, this F10 ELISA kit includes a set of calibrators. The calibrators are assayed at the same time as the samples and allow the operator to produce a calibration curve of optical density versus F10 concentration. The concentration of F10 in the samples is then determined by comparing the O.D. of the samples to the calibration curve.

PRINCIPLE

This F10 enzyme linked immunosorbent assay applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a polyclonal antibody specific for F10. Calibrators or samples are then added to the microtiter plate wells and F10 if present, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount of F10 present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for F10 are added to each well to “sandwich” the F10 immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, A and B substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain F10 and enzyme-conjugated antibody will exhibit a change in color. The enzyme-substrate reaction is terminated by addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm.

COMPONENTS

Reagents	Quantity
Microtiter Plate	96 wells
Calibrator 1 (0 pg/mL)	1
Calibrator 2 (50 pg/mL)	1
Calibrator 3 (100 pg/mL)	1
Calibrator 4 (250 pg/mL)	1
Calibrator 5 (500 pg/mL)	1
Calibrator 6 (1,000 pg/mL)	1
Enzyme Conjugate	1 x 10 mL
Substrate A	1 x 6 mL
Substrate B	1 x 6 mL
Stop Solution	1 x 6 mL
Wash Buffer (100X concentrate)	1 x 10 mL
Lysis Buffer Solution	1 x 3 mL

Note: The lysis buffer solution is used only when the sample is cell culture fluid & body fluid & tissue homogenate; If the sample is serum or blood plasma, then the lysis buffer solution is a superfluous reagent.

STORAGE

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube (SST) and allow samples to clot for 30 minutes before a centrifugation for 15 minutes at approximately 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20℃ or -80℃.

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000 x g at 4℃ within 30 minutes of collection. Store samples at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

Cell culture fluid and other biological fluids

Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

NOTE: Serum, plasma and cell culture fluid samples to be used within 7 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤2 months) or -80°C (≤6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES.**

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. 100 mL and 1 liter graduated cylinders.
4. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
5. 37℃ incubator.
6. Absorbent paper.
7. Distilled or de-ionized water
8. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log or semi-log, or log-logit as desired.
9. Tubes to prepare calibrator or sample dilutions.

Precautions

1. **Kamiya Biomedical Company** is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Please predict the concentration before assaying. If values for these are not within the range of the calibration curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g. antibody targets conformational isotope rather than linear isotope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
5. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.

6. Fresh samples without long term storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Bring all kit components and samples to room temperature (18-25°C) before use.

Dispense 5 µL of lysis buffer solution into 50 µL specimens, mix and stand for one hour (The proportion of lysis buffer and specimens shall be no less than 1:10). (**NOTE:** This step is required when the sample is cell culture fluid & body fluid & tissue homogenate; If the sample is serum or blood plasma, then this step should be skipped.)

Wash Solution

Dilute 10 mL of Wash Solution concentrate (100X) with 990 mL of de-ionized or distilled water to prepare 1,000 mL of Wash Solution (1X).

ASSAY PROCEDURE

It is recommended that all Calibrators and Samples be added in duplicate to the Microtiter Plate.

1. Secure the desired number of coated wells in the holder then add 50 µL of Calibrators or Samples to the appropriate well in the antibody pre-coated Microtiter Plate.
2. Add 100 µL of Conjugate to each well. Mix well. Mixing well in this step is important. Cover and incubate for 1 hour at 37°C.
3. Wash the microtiter plate using one of the specified methods indicated below:
4. Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of five washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.
5. Automated Washing: Wash plate five times with diluted wash solution (350-400 µL/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.
6. Add 50 µL substrate A and 50 µL substrate B to each well, subsequently. Cover and incubate for 15 minutes at 20-25°C. (Avoid sunlight.)
7. Add 50 µL of stop solution to each well. Mix well.
8. Read the optical density (OD) at 450 nm using a microtiter plate reader immediately.

CALCULATION OF RESULTS

1. This calibration curve is used to determine the amount of an unknown sample. Construct a calibration curve by plotting the average O.D. (450 nm) for each calibrator on the vertical (Y) axis against the concentration on the horizontal (X) axis, and draw a best fit curve through the points on the graph.
2. First, calculate the mean O.D. value for each calibrator and sample. All O.D. values are subtracted by the mean value of the blank control before result interpretation. Construct the calibration curve using graph paper or statistical software.
3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the calibration curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own calibration curve.
5. The sensitivity in this assay is 1.0 pg/mL.
6. This assay has high sensitivity and excellent specificity for detection of F10. No significant cross-reactivity or interference between F10 and analogues was observed.
   Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between F10 and all the analogues, therefore, cross reaction may still exist in some cases.

Disposal Note Safety

1. This kit contains materials with small quantities of sodium azide. sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of

water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin, and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

2. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

IMPORTANT NOTES

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag and store at 4°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all calibrators, controls, and samples be run in duplicate.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation time for all wells.
8. Cover or cap all reagents when not in use.
9. Do not mix or interchange different reagent lots from various kit lots.
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 2 hours of assay completion.
12. All residual wash 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 wells.
13. Because stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Also avoid contact between stabilized Chromogen and metal, otherwise color may develop.
14. Incomplete washing will adversely affect the test outcome. All washing must be performed with wash buffer provided.
15. Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.
16. After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15-30 seconds, and then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
17. Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
18. If using an automated washer, the operating instructions for washing equipment should be carefully followed.
19. Assay Procedure Preliminary notes: Do not mix reagents from different lots. It is recommended that assays be performed in duplicate. Calibrators and samples must be assayed at the same time. Avoid exposing the substrate to direct sunlight.

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