

LSBio™ Human F9 / Factor IX ELISA Kit

Catalog No. LS-F10414

User Manual

**Please Read the Manual Carefully
Before Starting your Experiment**



For research use only. Not approved for use in humans or for clinical diagnosis.

Human Coagulation Factor IX Total Antigen ELISA Kit

Catalog # LS-F10414

Strip well format. Reagents for up to 96 tests.

Rev: July 2014

INTENDED USE

This human coagulation Factor IX antigen assay is intended for the quantitative determination of total Factor IX antigen in human plasma. **For research use only.**

BACKGROUND

Factor IX (aka Christmas Factor) is a single-chain, 415 amino acid glycoprotein zymogen [1]. Factor IX is activated by either Factor XIa or the Factor VIIa complex [2]. Factor IXa converts Factor X to Factor Xa during the intrinsic pathway of the coagulation cascade. Factor IX is used to treat patients with hemophilia B, an X-linked bleeding disorder [3].

ASSAY PRINCIPLE

Human Factor IX will bind to the affinity purified capture antibody coated on the microtiter plate. Factor IX and IXa will react with the antibody on the plate. After appropriate washing steps, polyclonal anti-human Factor IX primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody, which is proportional to the total Factor IX present in the samples, is reacted with the secondary antibody. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human Factor IX. Color development is proportional to the concentration of Factor IX in the samples.

STANDARD CALIBRATION

Factor IX standard provided is calibrated against the WHO 4th International Standard for Factor IX, Plasma, Human distributed by NIBSC (09/172), South Mimms, Potters Bar, Hertfordshire, UK.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human Factor IX antibody, blocked and dried.
- **10X Wash Buffer:** 1 bottle of 50ml
- **Human Factor IX standard:** 1 vial lyophilized standard
- **Anti-human Factor IX primary antibody:** 1 vial lyophilized polyclonal antibody
- **Anti-goat horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

The secondary antibody must be stored at $\leq -70^{\circ}\text{C}$.

Store all other kit components at 4°C upon arrival.

Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once.

Store all other unused kit components at 4°C . This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- Blocking buffer (BB):** 3% BSA (w/v) in TBS
- 1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1000ng/ml standard solution.

Dilution table for preparation of human Factor IX standard:

Factor IX concentration (ng/ml)	Dilutions
100	900 μL (BB) + 100 μL standard from vial
50	500 μL (BB) + 500 μL (100ng/mL)
25	500 μL (BB) + 500 μL (50ng/mL)
10	600 μL (BB) + 400 μL (25ng/mL)
5	500 μL (BB) + 500 μL (10ng/mL)
2.5	500 μL (BB) + 500 μL (5ng/mL)
1	600 μL (BB) + 400 μL (2.5ng/mL)
0.5	500 μL (BB) + 500 μL (1ng/mL)
0.25	500 μL (BB) + 500 μL (0.5ng/mL)
0.1	600 μL (BB) + 400 μL (0.25ng/mL)
0	500 μL (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100 μL Factor IX standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μL wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures Factor IX antigen in the 0.1-100 ng/ml range. If the unknown is thought to have high Factor IX levels, dilutions may be made in blocking buffer. A 1:1,000 to 1:4,000 dilution for normal plasma is suggested for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100 μL to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μL wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 2 μL of conjugated secondary antibody in 10ml of blocking buffer and add 100 μL to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μL wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H₂SO₄ or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

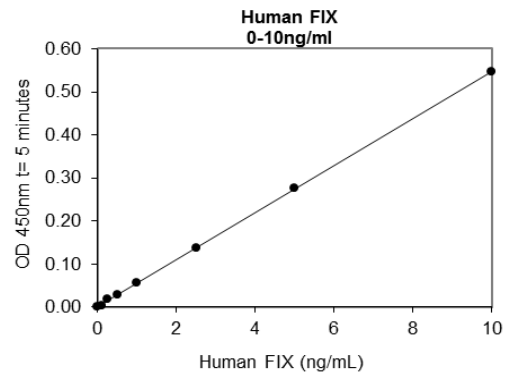
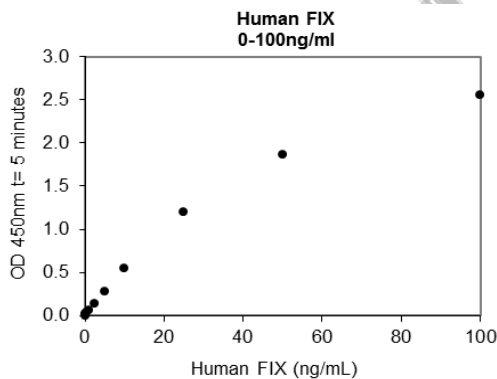
Measurement

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of Factor IX in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of Factor IX in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):



EXPECTED VALUES

The concentration of Factor IX in normal human plasma is about 5 µg/mL [4].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD₄₅₀: 0.049-0.055) and calculating the corresponding concentration. The MDD was 0.023ng/ml.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: These studies are currently in progress. Please contact us for more information.

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. DiScipio RG, *et al.*: Biochemistry. 1977, 16(4):698-706.
2. Soulier JP, Wartelle O, and Ménaché D: Rev Franc Etud Clin Biol. 1958, 3:263-267.
3. Roth DA, *et al.*: Blood. 2001, 98(13):3600-3606.
4. Thompson AR: Blood. 1986, 967(3):565-572.

Example of ELISA Plate Layout

96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml	
B	0	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml	
C												
D												
E												
F												
G												
H												

SAMPLE ONLY

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Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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