Catalog # HFIXKT-TOT

Total Human Coagulation Factor IX Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This human coagulation Factor IX antigen assay is intended for the quantitative determination of total Factor IX antigen in human plasma.

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.

Lot 1211L: 1000 ng = 0.226 IU

REAGENTS PROVIDED

- **96-well microtiter strip plate:**
  8X12 removable well strips containing affinity purified anti-human Factor IX antibody dried and blocked on the surface
- **10X Wash Buffer:**
  1 bottle of 50mL; bring to 1X using DI water
- **Human Factor IX standard:**
  1 vial of lyophilized standard
- **Anti-human Factor IX primary antibody:**
  1 vial of lyophilized polyclonal antibody
- **Anti-goat secondary antibody:**
  1 vial of concentrated HRP-labeled antibody
- **TMB substrate solution:**
  1 bottle of 10mL solution

STORAGE AND STABILITY

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 0-10µL and 200-1000µL
- 12-channel pipette covering 30-300µL
- Paper towels or kimwipes
- 50mL tubes, 1.5mL centrifuge tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

WARNINGS

Warning – Avoid skin and eye contact when using TMB substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

PRECAUTIONS

- **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.
- **DO NOT** pipette reagents by mouth.
- Always pour TMB substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the TMB substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **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 in TBS
- **Wash buffer concentrate:** The wash buffer supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

SPECIMEN COLLECTION

The assay measures total human Factor IX in the 0.1-100 ng/mL range. Samples giving human Factor IX levels above 100ng/mL should be diluted in blocking buffer before use. A 1:500 to 1:1,000 dilution for plasma is suggested for best results.

ASSAY PROCEDURE

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

Preparation of Standard:
Reconstitute standard as directed on the vial to give a 1,000ng/mL solution.

Dilution table for preparation of human Factor IX standards:

Factor IX concentration (ng/mL)	Dilutions
100	900µL (BB) + 100µL (1000ng/mL)
50	500µL (BB) + 500µL (100ng/mL)
25	500µL (BB) + 500µL (50ng/mL)
10	600µL (BB) + 400µL (25ng/mL)
5	500µL (BB) + 500µL (10ng/mL)
2.5	500µL (BB) + 500µL (5ng/mL)
1	600µL (BB) + 400µL (2.5ng/mL)
0.5	500µL (BB) + 500µL (1ng/mL)
0.25	500µL (BB) + 500µL (0.5ng/mL)
0.1	600µL (BB) + 400µL (0.25ng/mL)
0	500µL (BSA) 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. Add 100µL 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 300µL wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

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

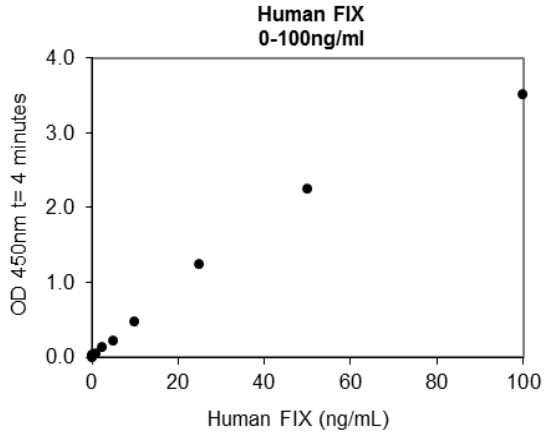
Secondary Antibody Addition:
Dilute 1µL into 10mL blocking buffer and add 100µL to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µL wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

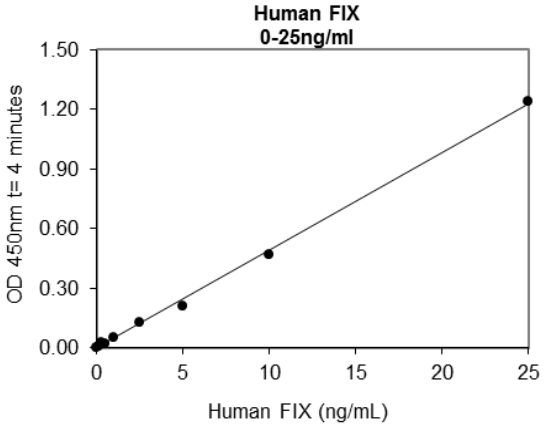
Substrate Incubation:
Add 100µL TMB substrate to all wells and shake plate for 2-7 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µL of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance values at 450nm. For best results read plate immediately

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}).

Assay Calibration:
Plot A_{450} against the amount of human Factor IX in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total human Factor IX in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





EXPECTED VALUES

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

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 or from 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 Kit Plate Layout:
96 Well Plate

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

Standards: 22 Wells
Samples: 74 Wells