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Human Total Factor IX ELISA Kit

Catalog No: CS219A
CS219B

Size: 1 x 96 tests
5 x 96 tests

Sensitivity:	0.042 ng/ml
Specificity:	Human Factor IX antigen
Range:	0.10 - 100 ng/ml
Sample Type:	Human plasma
Cross-Reactivity:	Pooled normal plasma from mouse, rat, pig, dog, cynomolgus monkey and rhesus monkey were assayed and no significant cross-reactivity was observed.

Background: Factor IX (aka Christmas Factor) is a single-chain, 415 amino acid glycoprotein zymogen. Factor IX is activated by either Factor XIa or the Factor VIIa complex. 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.

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.

Reagents Provided:

Description	Quantity
CS219A – P. 96-well microtiter strip plate coated with anti-Human Factor IX antibody, blocked and dried on well surfaces	1 plate: 96 wells (12 strips x 8 wells)
CS219A - A. Wash Buffer Concentrate (10x)	1 bottle, 50 mL
CS219A - B. Human Factor IX Standard, lyophilized	1 vial
CS219A - C. Anti-Human Factor IX primary antibody, lyophilized polyclonal antibody	1 vial
CS219A - D. Horseradish peroxidase-Anti-goat secondary antibody, concentrated	1 vial
CS219A - E. TMB substrate solution	1 bottle, 10 ml

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

Cell Sciences®
65 Parker Street
Unit 11
Newburyport, MA 02021

Toll Free: 888 769-1246
Phone: 978-572-1070
Fax: 978-992-0298

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Web Site: www.cellsciences.com

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Storage and Stability:

The secondary antibody CS219-D must be stored at -80°C. All kit components are stored at 2-8°C upon receipt. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -80°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 2-8°C. Kit should be used no later than the expiration date.

Reagents and Equipment Required:

- Pipettes covering 0-10 µl and 200-1000 µl, and tips
- 12-channel pipette covering 30-300µl
- Paper towels or laboratory wipes
- Polypropylene conical 50 ml tubes, 1.5 ml flip-cap tubes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)
- Deionized or distilled water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm
- Automatic plate washer or wash bottle

Warnings:

Warning – Avoid skin and eye contact when using TMB One 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 substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the 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:** 0.1 M Tris 0.15 M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer concentrate:** Dilute 50 ml of 10X wash buffer with 450 ml deionized water

Specimen Collection:

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or aliquot and store at $\leq$ - 20°C. Avoid repeated freeze-thaw cycles.

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Assay Procedure: Allow microtiter strips and assay components to warm to room temperature for 30 minutes. Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:
Reconstitute Standard by adding **1.0 ml of blocking buffer** directly to the vial and agitate gently to completely dissolve contents. This will result in a **1000 ng/ml Standard solution**.

Table 1: Dilution table for preparation of Human Factor IX Standards:

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 (50 ng/ml)
10	600 µl (BB) + 400 µl (25 ng/ml)
5	500 µl (BB) + 500 µl (10 ng/ml)
25	500 µl (BB) + 500 µl (5 ng/ml)
1	600 µl (BB) + 400 µl (2.5 ng/ml)
0.5	500 µl (BB) + 500 µl (1.0 ng/ml)
0.25	500 µl (BB) + 500 µl (0.5 ng/ml)
0.1	600 µl (BB) + 400 µl (0.25 ng/ml)
0	500 µl (BB) Zero point to determine background

NOTE: Dilutions for the standard curve must be made and applied to the plate immediately.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100 µl of Factor IX standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipes.

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

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Primary Antibody Addition:
Reconstitute primary antibody by adding **10 ml blocking buffer** to vial. Agitate gently to completely dissolve contents. Add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.

Streptavidin-HRP Addition:
Briefly centrifuge vial before opening. **Dilute 2 µl** of HRP-conjugated secondary antibody into 10 ml blocking buffer and add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.

Substrate Incubation:
Add 100 µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different intensities of blue. Quench reaction by adding 50 µl of 1N H_2SO_4 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 and read plate immediately.

Measurement:
Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450 nm. 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 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.

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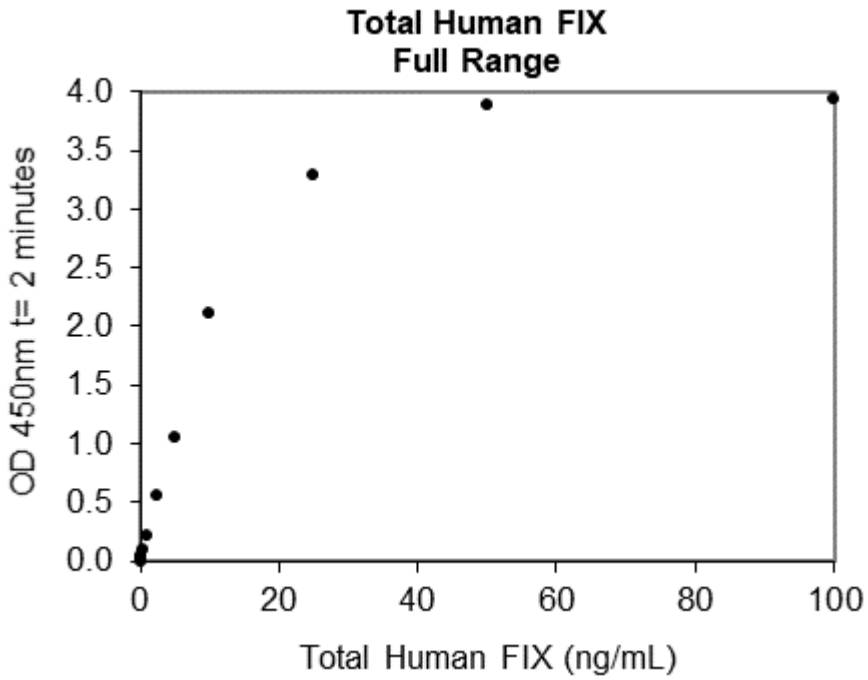
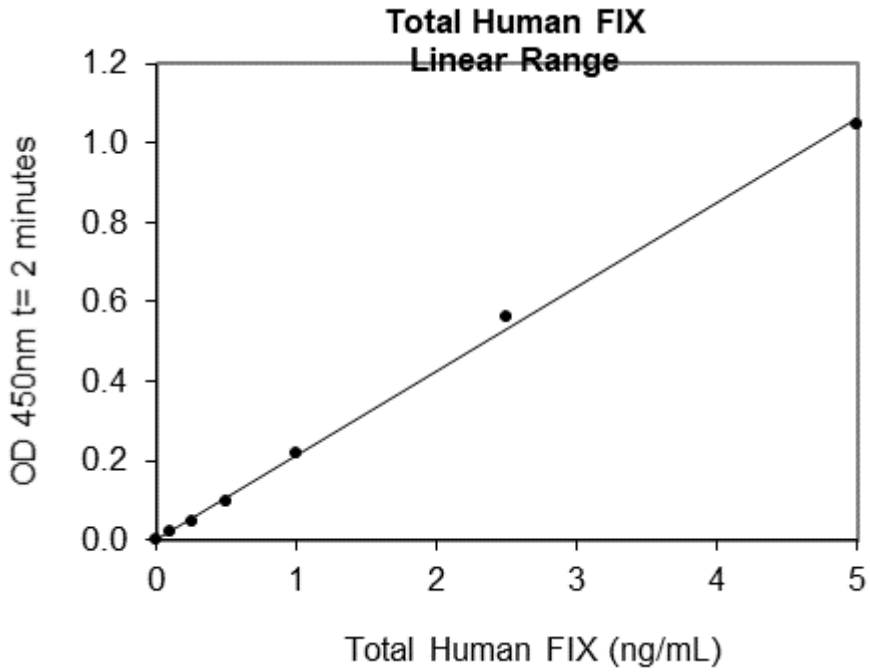
A typical standard curve. **(EXAMPLE ONLY, DO NOT USE)**





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Expected Values: The average concentration of Factor IX in normal Human plasma is about 5 μg/ml.

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 OD_{450}: 0.054-0.066) and calculating the corresponding concentration. The MDD was 0.042 ng/ml.

Specificity: This assay recognizes total human Factor IX. Pooled normal plasma from mouse, rat, pig, dog, cyno monkey, and rhesus monkey were tested, and no significant cross-reactivity was observed.

Intra-assay Precision: 3 samples of known concentration were tested 20 times on 1 plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.226	1.56	5.33
Standard Deviation	0.02	0.095	0.362
CV (%)	8.84	6.09	6.80

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Inter-assay Precision: Three samples of known concentration were tested in 10 independent assays.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	0.811	3.07	16.2
Standard Deviation	0.064	0.133	1.19
CV (%)	7.93	4.34	7.38

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in depleted plasma was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	0.4	1.95	8.58	16.2
Average % Recovery	100	98	114	108
Range (%)	87-119%	89-113%	107-119%	106-111%

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Linearity: To assess the linearity of the assay, pooled citrated human plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % Expected	100	91	91	94
Recovery	94-103%	89-94%	88-93%	91-98%

Disclaimer: This information is believed to be correct but does not claim to be all-inclusive and should 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.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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