Human Total Factor VIII ELISA Kit

Catalog # IHUFVIIIKTT
Strip well format. Reagents for up to 96 tests.
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INTENDED USE

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

BACKGROUND

Factor VIII (aka Factor VIII:C or Antihemophilic Globulin) is a glycoprotein zymogen that circulates in a stabilized non-covalent complex with von Willebrand Factor (vWF) [1]. Following activation by thrombin or Factor Xa, Factor VIIIa dissociates from vWF and catalyzes the activation of Factor X by Factor IXa in the amplification phase of coagulation [2]. Factor VIIIa activity is quickly decreased by spontaneous dissociation and proteolytic degradation by activated Protein C, Factor Xa and Factor IXa [3]. Hemophilia A is caused by mutations in the Factor VIII gene; a majority of patients have decreased Factor VIII plasma levels while 5% of patients have normal levels of nonfunctioning protein [4].

ASSAY PRINCIPLE

Human Factor VIII will bind to the capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled anti-human Factor VIII primary antibody binds to the captured protein. Excess primary antibody is washed away, and bound antibody is reacted with peroxidase conjugated streptavidin. 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 plasma. Color development is proportional to the concentration of Factor VIII in the samples.

STANDARD CALIBRATION

The Factor VIII level in the human plasma standard provided is calibrated against a secondary standard that is referenced to the WHO or ISTH International Standard. **See C of A for lot specific Calibration of Standard.**

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human factor VIII antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human Factor VIII standard:** 1 vial lyophilized plasma
- **Anti-human Factor VIII primary antibody:** 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary 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)

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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 or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°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 according to the C of A. Agitate gently to completely dissolve contents.

Dilution table for preparation of human Factor VIII standard:

Factor VIII concentration (IU/ml)	Dilutions
1.05	From vial
0.525	500µl (BB) + 500µl (1.0 IU/ml)
0.2625	500µl (BB) + 500µl (0.525 IU/ml)
0.1313	500µl (BB) + 500µl (0.2625 IU/ml)
0.0656	500µl (BB) + 500µl (0.1313 IU/ml)
0.0328	500µl (BB) + 500µl (0.0656 IU/ml)
0.0164	500µl (BB) + 500µl (0.0328 IU/ml)
0.0082	500µl (BB) + 500µl (0.0164 IU/ml)
0.0041	500µl (BB) + 500µl (0.0082 IU/ml)
0.0021	500µl (BB) + 500µl (0.0041 IU/ml)
0	500µl (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 100µl Factor VIII 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.

NOTE: The assay measures Factor VIII antigen in the 0.0021-1.05 IU/ml range. 1:50 and 1:100 dilutions for normal plasma, or 1:4 and 1:8 dilutions for Haemophiliac plasma, are 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 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.

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Streptavidin-HRP Addition

Briefly centrifuge vial before opening. **See C of A for lot specific dilution instructions.** Add 100µl of your dilution 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 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl 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.

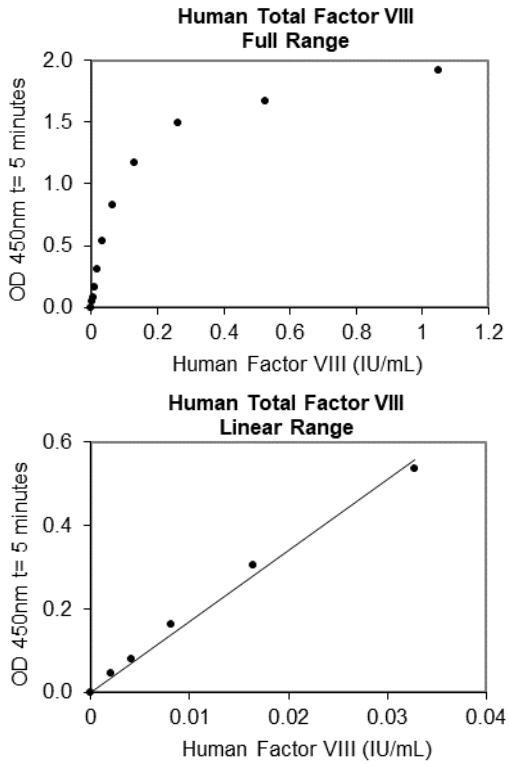
Measurement

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 VIII 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 VIII 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 average normal plasma level of Factor VIII is defined as 1.0 IU/ml and the normal range is 0.4-1.8 IU/ml [5]. Hemaophilia A patients are classified by the following Factor VIII levels: 0.05-0.25 IU/ml = mild, 0.01-0.05 IU/ml = moderate, and <0.01 IU/ml = severe [6].

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 and calculating the corresponding concentration. **See C of A for lot specifications.**

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.

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

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 of or contact with the above product.

REFERENCES

1. Hoyer LW: Blood 1981, 58(1):1-13.
2. Lollar P, *et al.*: Methods Enzymol. 1993, 222:128-43.
3. Lenting PJ, *et al.*: Blood 1998, 92(11):3983-96.
4. Amano K, *et al.*: Blood 1998, 91(2):538-48.
5. Kasper CK: Haemophilia 2000, 6 (s1):13-27.
6. Hedner U, *et al.*: Hematology 2000, 1:241-265.

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	0.0021 IU/ml	0.0043 IU/ml	0.0086 IU/ml	0.0172 IU/ml	0.0344 IU/ml	0.0688 IU/ml	0.1375 IU/ml	0.275 IU/ml	0.55 IU/ml	1.1 IU/ml	
B	0	0.0021 IU/ml	0.0043 IU/ml	0.0086 IU/ml	0.0172 IU/ml	0.0344 IU/ml	0.0688 IU/ml	0.1375 IU/ml	0.275 IU/ml	0.55 IU/ml	1.1 IU/ml	
C												
D												
E												
F												
G												
H												

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