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# ab272771

## Human FVIII ELISA kit (total FVIII antigen)

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For the quantitative determination of total FVIII in human plasma (citrate, EDTA).

This product is for research use only and is not intended for diagnostic use.

## Table of Contents

1. Overview	1
2. Protocol Summary	2
3. Precautions	3
4. Storage and Stability	3
5. Limitations	4
6. Materials Supplied	4
7. Materials Required, Not Supplied	5
8. Technical Hints	5
9. Reagent Preparation	6
10. Sample Preparation	6
11. Sample Dilution	6
12. Preparation of Standards	7
13. Assay Procedure	8
14. Typical Results	10
15. Performance Characteristics	11
16. Specificity	12
17. Notes	13
<b>Technical Support</b>	<b>14</b>

# 1. Overview

The sensitive quantitative measurement of total human Factor VIII antigen in plasma samples is easily performed with Human FVIII ELISA kit (total FVIII antigen) (ab272771).

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.

Hemophilia A patients are classified by the following Factor VIII levels:

0.05 - 0.25 IU/ml = mild

0.01 - 0.05 IU/ml = moderate

less than 0.01 IU/ml = severe

The assay measures total human Factor VIII in the 0.0016-0.84 IU/ml range. Samples giving human Factor VIII levels above 0.84 IU/ml should be diluted in blocking buffer before use. 1:50 and 1:100 dilutions for normal plasma, or 1:4 and 1:8 dilutions for Hemophilic plasma, are suggested for best results.

Human Factor VIII will bind to the affinity purified 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. All reagents and standards are provided in these ELISA kits.

## 2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.



Add 100  $\mu\text{L}$  standard or sample to appropriate wells and shake plate at 300 rpm for 30 mins. Wash wells three times with 300  $\mu\text{L}$  wash buffer. Remove excess wash by gently tapping plate on paper towel.



Add 100  $\mu\text{L}$  of primary antibody to each well and shake plate at 300 rpm for 30 mins. Wash wells three times with 300  $\mu\text{L}$  wash buffer. Remove excess wash by gently tapping plate on paper towel.



Add 100  $\mu\text{L}$  Streptavidin-HRP to each well and shake plate at 300 rpm for 30 mins. Wash wells three times with 300  $\mu\text{L}$  wash buffer. Remove excess wash by gently tapping plate on paper towel.



Add 100  $\mu\text{L}$  TMB Substrate Solution to each well and shake plate for 2-10 mins.



Stop reaction with 50  $\mu\text{L}$   $\text{H}_2\text{SO}_4$  or HCl stop solution, then measure the absorbance in all wells at 450nm.

### 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

### 4. Storage and Stability

**Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

Item	Quantity	Storage Condition
ELISA Plate	1 x 96 tests	+4°C
10X Wash Buffer	1 x 50 mL	+4°C
Human FVIII Standard Lyophilized Vial	1 vial	+4°C
Anti-Human FVIII Primary Antibody Lyophilized Vial	1 vial	+4°C
Streptavidin-HRP Secondary Reagent	1 vial	+4°C
TMB Substrate	1 x 10 mL	+4°C

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate plate shaker capable of 300 rpm uniform horizontal 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<sub>2</sub>SO<sub>4</sub> or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

## 8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.

## 9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

### 9.1 TBS buffer:

Prepare 0.1M Tris, 0.15M NaCl, pH 7.4.

### 9.2 Blocking buffer (BB):

Prepare 3% BSA (w/v) in TBS.

### 9.3 1X Wash Buffer:

Dilute 50 mL of 10X wash buffer concentrate with 450 mL of deionized water.

## 10. Sample Preparation

- Use human plasma (citrate, EDTA) samples with this assay.
- Centrifuge for 15 mins at 1000 *xg* within 30 mins of collection.
- Assay immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freezing and thawing.

## 11. Sample Dilution

- The assay measures Factor VIII antigen in the 0.0016-0.84 IU/ml range.
- 1:50 and 1:100 dilutions for normal plasma, or 1:4 and 1:8 dilutions for Hemophiliac plasma, are suggested for best results.

## 12. Preparation of Standards

- Dilutions for the standard curve and zero standard must be made and applied to the plate immediately.

- 12.1** The vial has a concentration identified, reconstitute the Human FVIII protein lyophilized standard by adding 1 mL deionized water directly to the vial, agitate gently to completely dissolve contents. This will result in the identified concentration plasma standard.
- 12.2** Then perform a 2-fold serial dilution using blocking buffer (BB). Standard #11 contains no protein and is the blank control.

The following example is for reference (Example Range: 0.0017-0.85 IU/ml):

Factor VIII concentration (IU/ml)	Dilutions
0.85	From vial
0.425	500µl (BB) + 500µl (0.85 IU/ml)
0.2125	500µl (BB) + 500µl (0.425 IU/ml)
0.1063	500µl (BB) + 500µl (0.213 IU/ml)
0.0531	500µl (BB) + 500µl (0.106 IU/ml)
0.0266	500µl (BB) + 500µl (0.053 IU/ml)
0.0133	500µl (BB) + 500µl (0.027 IU/ml)
0.0066	500µl (BB) + 500µl (0.013 IU/ml)
0.0033	500µl (BB) + 500µl (0.007 IU/ml)
0.0017	500µl (BB) + 500µl (0.003 IU/ml)
0	500µl (BB) Zero point to determine background

## 13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Vigorously shake plate (300 rpm) at each step of the assay.

### 13.1 Standard and Unknown Addition.

- 13.1.1 Remove microtiter plate from bag and add 100  $\mu$ L Factor VIII standards (in duplicate) and unknowns to wells.
- 13.1.2 Carefully record position of standards and unknowns. Shake plate at 300 rpm for 30 mins.
- 13.1.3 Wash wells three times with 300  $\mu$ L wash buffer. Remove excess wash by gently tapping plate on paper towel.  
**Δ Note:** The assay measures Factor VIII antigen in the 0.0017-0.85 IU/ml range. 1:50 and 1:100 dilutions for normal plasma, or 1:4 and 1:8 dilutions for Hemophilic plasma, are suggested for best results.

### 13.2 Primary Antibody Addition.

- 13.2.1 Reconstitute primary antibody by adding 10 mL of blocking buffer directly to the vial and agitate gently to completely dissolve contents.
- 13.2.2 Add 100  $\mu$ L to all wells. Shake plate at 300 rpm for 30 mins.
- 13.2.3 Wash wells three times with 300  $\mu$ L wash buffer. Remove excess wash by gently tapping plate on paper towel.

### 13.3 Streptavidin-HRP Addition.

- 13.3.1 Briefly centrifuge vial before opening.
- 13.3.2 Dilute 2.5  $\mu$ L of HRP conjugated streptavidin into 2.5 mL blocking buffer to generate a 1:1,000 dilution.
- 13.3.3 Add 0.2 mL of the 1:1,000 dilution to 9.8 mL of blocking buffer to generate a 1:50,000 dilution.
- 13.3.4 Add 100  $\mu$ L of the 1:50,000 dilution to all wells. Shake plate at 300 rpm for 30 mins.
- 13.3.5 Wash wells three times with 300  $\mu$ L wash buffer. Remove excess wash by gently tapping plate on paper towel.

### 13.4 Substrate Incubation.

- 13.4.1 Add 100  $\mu$ L TMB substrate to all wells and shake plate for 2 to 10 mins. Substrate will change from colorless to different strengths of blue.

13.4.2 Quench reaction by adding 50  $\mu\text{L}$  of 1N  $\text{H}_2\text{SO}_4$  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.

### **13.5 Measurement.**

13.5.1 Set the absorbance at 450 nm in a microtiter plate spectrophotometer.

13.5.2 Measure the absorbance in all wells at 450 nm.

13.5.3 Subtract zero point from all standards and unknowns to determine corrected absorbance ( $A_{450}$ ).

### **13.6 Calculation of Results.**

13.6.1 Plot  $A_{450}$  against the amount of Factor VIII in the standards.

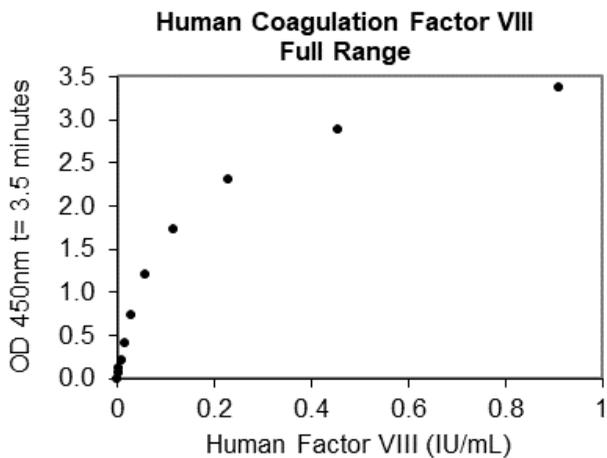
13.6.2 Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve.

13.6.3 Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit.

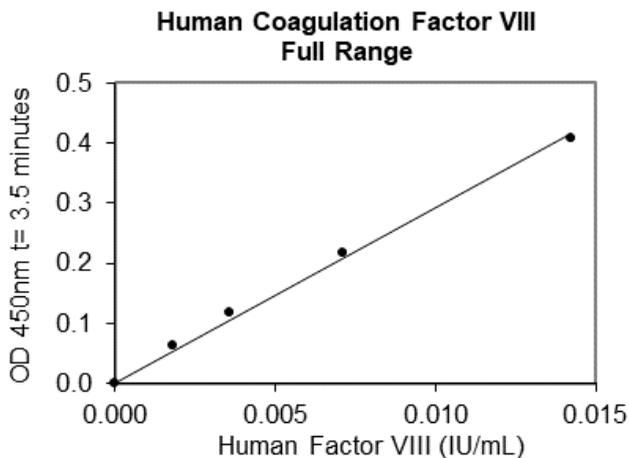
13.6.4 The amount of Factor VIII in the unknowns can be determined from this curve.

13.6.5 If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

## 14. Typical Results



**Figure 1.** A typical standard curve. Example only.



**Figure 2.** A typical standard curve. Example only.

## 15. Performance Characteristics

### 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. Hemophilia 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.

### Sensitivity:

The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of eighteen zero standard replicates (range OD450: 0.064-0.072) and calculating the corresponding concentration.

The MDD was 0.00024 IU/ml.

### Intra-assay Precision:

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.0124	0.0370	0.2125
Standard Deviation	0.0004	0.0018	0.0170
CV (%)	3.51	4.80	7.99

### Linearity:

To assess the linearity of the assay, 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 % of expected	95	96	90	100
Range	92-99%	93-99%	89-94%	98-103%

## 16. Specificity

This assay recognizes total human Factor VIII.

Pooled normal plasma from mouse, rat, rabbit, pig, horse, guinea pig, dog, and sheep were assayed, and no significant cross-reactivity was observed. Pooled normal plasma from cynomolgus monkey, rhesus monkey and baboon were assayed, and significant cross-reactivity was observed.

## 17. Notes

## Technical Support

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