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Cyno Monkey Coagulation Factor VIII Total Antigen ELISA Kit

Catalog # CYFVIIIKT-TOT

Strip well format. Reagents for up to 96 tests.

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INTENDED USE

This cynomolgus macaque (Macaca fascicularis) monkey coagulation Factor VIII antigen assay is intended for the quantitative determination of total Factor VIII antigen in cyno 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

Cyno Factor VIII will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled anti-cyno 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 cyno plasma. Color development is proportional to the concentration of Factor VIII in the samples.

STANDARD CALIBRATION

The Factor VIII level in the cyno plasma standard provided is calibrated against a human plasma secondary standard that is referenced to the WHO or ISTH International Standard.

Lot 315L: 1.42 IU/ml

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-cyno factor VIII antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Cyno Factor VIII standard:** 1 vial lyophilized plasma
- **Anti-cyno Factor VIII primary antibody:** 1 vial concentrated 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 standards may be stored at -80°C for later use. Do not freeze-thaw the standard 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 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 by adding 1ml of water directly to the vial and agitate gently to completely dissolve contents. This will result in a 1.42 IU/ml plasma standard.

Dilution table for preparation of cyno Factor VIII standard:

Factor VIII concentration (IU/ml)	Dilutions
1.42	From vial
0.71	500µl (BB) + 500µl (1.42 IU/ml)
0.355	500µl (BB) + 500µl (0.71 IU/ml)
0.178	500µl (BB) + 500µl (0.355 IU/ml)
0.089	500µl (BB) + 500µl (0.178 IU/ml)
0.044	500µl (BB) + 500µl (0.089 IU/ml)
0.022	500µl (BB) + 500µl (0.044 IU/ml)
0.011	500µl (BB) + 500µl (0.022 IU/ml)
0.006	500µl (BB) + 500µl (0.011 IU/ml)
0.003	500µl (BB) + 500µl (0.006 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 0-1.42 IU/ml range. 1:8 and 1:16 dilutions for normal plasma, or 1:4 and 1:8 dilutions for Haemophiliac plasma, are suggested for best results.

Primary Antibody Addition

Briefly centrifuge vial before opening. Prepare the primary antibody by adding 2µl of concentrated antibody into 10ml of blocking buffer and mix well. 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.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.2ml of the 1:1,000 dilution to 9.8ml of blocking buffer to generate a 1:50,000 dilution. Add 100µl of the 1:50,000 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 2-5 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.

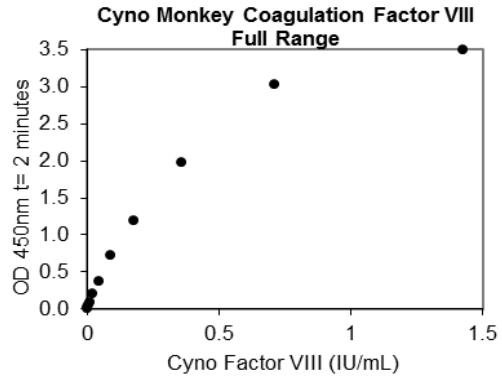
Measurement

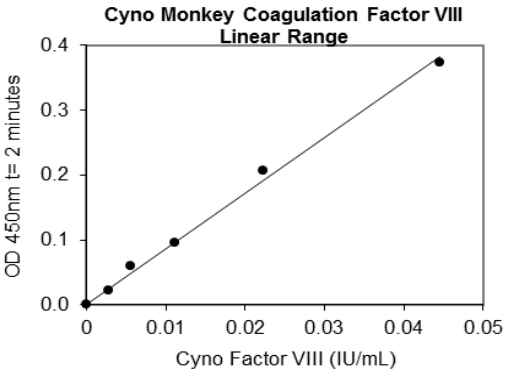
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

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 human 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]. Normal values of Factor VIII in cyno plasma have not been conclusively determined but are believed to be similar to human plasma.

PERFORMANCE CHARACTERISTICS

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 OD_{450}: 0.064-0.074) and calculating the corresponding concentration. The MDD was 0.00035 IU/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: This assay recognizes total cyno Factor VIII. Pooled normal plasma from mouse, rat, rabbit, pig, horse, guinea pig, dog, and sheep was assayed and no significant cross-reactivity was observed. Pooled normal plasma from human, rhesus monkey and baboon was assayed and significant cross-reactivity was observed.

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.

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	0.003 IU/ml	0.006 IU/ml	0.011 IU/ml	0.022 IU/ml	0.044 IU/ml	0.089 IU/ml	0.178 IU/ml	0.355 IU/ml	0.71 IU/ml	1.42 IU/ml	
B	0	0.003 IU/ml	0.006 IU/ml	0.011 IU/ml	0.022 IU/ml	0.044 IU/ml	0.089 IU/ml	0.178 IU/ml	0.355 IU/ml	0.71 IU/ml	1.42 IU/ml	
C												
D												
E												
F												
G												
H												

REFERENCES

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