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Human Coagulation Factor V Total Antigen ELISA Kit

Catalog # HFVKT-TOT

Strip well format. Reagents for up to 96 tests.

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INTENDED USE

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

BACKGROUND

Factor V (aka proaccelerin or labile factor) is a 2224 amino acid single chain glycoprotein [1]. Factor V is activated to Factor Va by thrombin. Factor Va binds to Factor Xa and acts as a cofactor in accelerating the activation of prothrombin to thrombin [2]. A genetic Factor V R506Q mutation has been shown to result in a resistance to activated protein C leading to venous thrombosis [3].

ASSAY PRINCIPLE

Human Factor V will bind to the capture monoclonal antibody coated on the microtiter plate. Factor V and Va will react with the antibody on the plate. After appropriate washing steps, biotinylated primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with horseradish peroxidase conjugated streptavidin. 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 V. Color development is proportional to the concentration of Factor V in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 12x8) containing anti-human Factor V antibody, blocked and dried.
- **20X Wash buffer:** 2 bottles of 30ml
- **10X Diluent:** 1 bottle of 30ml
- **Human Factor V standard:** 1 vial lyophilized standard
- **Anti-human Factor V primary antibody:** 1 vial concentrated antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **Chromogen substrate solution:** 1 bottle of 8ml
- **Stop solution:** 1 bottle of 12ml

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Primary antibody and streptavidin conjugate should be stored at -20°C upon arrival. Store the standard at -20°C after reconstituting. 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

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

- **1X Diluent:** 10X Diluent may contain precipitate. Warm to redissolve before use. Dilute 30ml of 10X diluent concentrate with 270ml of deionized water.
- **1X Wash buffer:** 20X Wash buffer may contain precipitate. Warm to redissolve before use. Dilute 60ml of 20X wash buffer concentrate with 1140ml of deionized water.

SAMPLE COLLECTION

Collect plasma using EDTA, heparin, 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 **3.5ml of diluent** directly to the vial and agitate gently to completely dissolve contents. This will result in a 60ng/ml standard solution.

Dilution table for preparation of human Factor V standard:

Factor V concentration (ng/ml)	Dilutions
60	From standard vial
30	125µl Diluent + 125µl (60ng/ml)
15	125µl Diluent + 125µl (30ng/ml)
7.5	125µl Diluent + 125µl (15ng/ml)
3.75	125µl Diluent + 125µl (7.5ng/ml)
1.875	125µl Diluent + 125µl (3.75ng/ml)
0.938	125µl Diluent + 125µl (1.875ng/ml)
0	125µl Diluent 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 50µl Factor V standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells six times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures Factor V antigen in the 0.938-60 ng/ml range. If the unknown is thought to have high Factor V levels, dilutions may be made in diluent. A 1:800 dilution for normal human plasma is suggested for best results.

Primary Antibody Addition

Briefly centrifuge vial before opening. Dilute 100µl of primary antibody in 6 ml of diluent and add 50µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells six 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 50µl of HRP conjugated streptavidin in 5ml of diluent and add 50µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells six times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 50µl TMB substrate to all wells and shake plate for 5-20 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 0.5N 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.

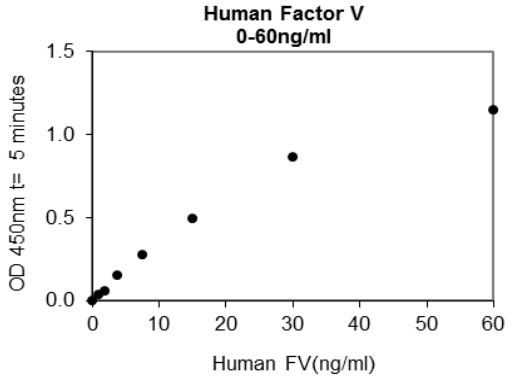
Measurement

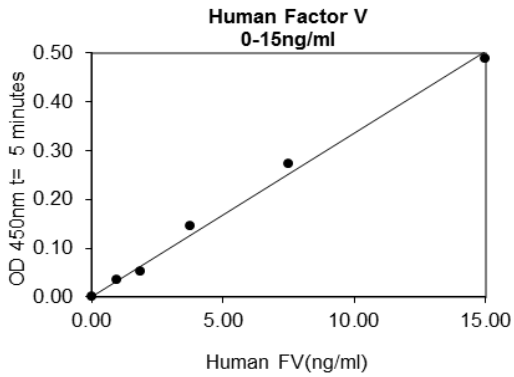
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of Factor V 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 V 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 concentration of Factor V in normal human plasma ranges from 4 to 14 µg/ml with an average value of 7 ug/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 of or contact with the above product.

REFERENCES

1. Jenny RJ, *et al.*: PNAS. 1987, 84:4846-50.
2. Camire RM and Bos MHA: J Thromb Haemost. 2009, 7:1951-1961.
3. Zoller B, *et al.*: J Clin Invest. 1994, 94:2521-2524.
4. Tracy PB, *et al.*: Blood. 1982, 60:59-63.

Example of ELISA Plate Layout
96 Well Plate: 16 Standard wells, 80 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0										
B	0.938 ng/ml	0.938 ng/ml										
C	1.875 ng/ml	1.875 ng/ml										
D	3.75 ng/ml	3.75 ng/ml										
E	7.5 ng/ml	7.5 ng/ml										
F	15 ng/ml	15 ng/ml										
G	30 ng/ml	30 ng/ml										
H	60 ng/ml	60 ng/ml										