



Factor V Antibody Pair [HRP]

Catalog Number NBP2-42116

Novus kits are guaranteed for 6 months from date of receipt

INTRODUCTION

Factor V (formerly referred to as accelerator globulin and labile factor) is a large glycoprotein (320 kDa) that is produced in the liver. The gene that encodes factor V (F.V) is located on chromosome 1. A congenital deficiency of F.V is a hemorrhagic disorder inherited as an autosomal recessive disease. The concentration of F.V in plasma is typically 10 µg/ml. F.V is a pro-cofactor that is activated through limited proteolysis by thrombin, or by activated factor X in the presence of phospholipid surface. Other physiologic activators of F.V include plasmin, neutrophil elastase and platelet calpain. The activated cofactor (F.Va) is an essential component of the prothrombin activator complex, which consists of F.Va, activated factor X, calcium and anionic phospholipid surface. The intact prothrombinase complex activates prothrombin to thrombin at a rate 300,000-fold greater than activated factor X alone. In a positive feedback loop, the thrombin generated accelerates its own generation by activating more F.V to F.Va. Thrombin also acts to down-regulate F.Va indirectly by activating Protein C, which inactivates F.Va cofactor activity.

PRINCIPLE OF SANDWICH ELISA

Affinity-purified antibody to F.V is coated onto the wells of a microtiter plate. Any remaining binding sites on the plastic wells are blocked with an excess of bovine serum albumin. The plates are washed and plasma or other fluids containing F.V are applied. The coated antibody will capture the F.V in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to F.V is added to the plate to bind to the captured F.V. After washing the plate to remove unbound conjugated antibody, the peroxidase activity is expressed by incubation with o-phenylenediamine (OPD). After a fixed development time, the reaction is quenched with the addition of H₂SO₄ and the color produced is quantified using a microplate reader. The color generated is proportional to the concentration of F.V present in the sample.

KIT COMPONENTS

NBP2-42116A: Capture Antibody, 1 x 500 µl. Supplied as a liquid in 50% glycerol. Affinity purified Factor V polyclonal antibody for coating ELISA plates.

NBP2-42116B: Detection Antibody, 1 x 500 µl. Supplied as a liquid in 50% glycerol. HRP-conjugated Factor V Pab for detection of captured Factor V.

STORAGE AND STABILITY

For long-term storage, aliquot and store at -20°C. Aliquots are stable for at least 12 months at -20°C. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Further dilutions can be made in assay buffer.

MATERIALS REQUIRED BUT NOT SUPPLIED

Coating Buffer (50 mM Carbonate): For 1 liter: 1.59 g Na₂CO₃ and 2.93 g NaHCO₃. Adjust pH to 9.6. Store at 4°C up to 1 month.

PBS (base for wash buffer and blocking buffer): For 1 liter: 8.0 g NaCl, 1.15 g Na₂HPO₄, 0.2 g KH₂PO₄, 0.2 g KCl, up to 1 liter. Adjust pH to 7.4. Store at 4°C up to 1month; discard if there is evidence of microbial growth.

Sample Diluent (HBS-BSA-Tween): 5.95 g HEPES (free acid), 1.46 g NaCl, 2.5 g Bovine Serum Albumin (RIA grade), dissolved in 200 ml water. Add 0.25 ml Tween-20. Adjust pH to 7.2 with NaOH, then make up to a final volume of 250ml with water. Aliquot and store frozen at -20°C.

Wash Buffer: PBS-Tween (0.1% v/v): For 1 liter of PBS, add 1 ml of Tween-20. Check that pH is 7.4. Store at 4°C up to 1 week.

Blocking Buffer: PBS-BSA (1%, w/v). Dissolve 2.5 g BSA in 200 ml of PBS. Adjust to pH 7.4, if required, then make up to 250 ml with PBS. Aliquot and store frozen at -20°C.

Substrate Buffer: Citrate-Phosphate buffer pH 5.0. 2.6 g Citric acid and 6.9 g Na₂HPO₄. Make up to final volume of 500 ml with water. Store at 4°C up to 1month

OPD Substrate (O-Phenylenediamine.2HCl): 5 mg tablets. Make up immediately before use. Dissolve 5 mg OPD in 12 ml substrate buffer then add 12 µl 30% H₂O₂. Note: Toxic!

Stopping Solution (2.5 M H₂SO₄): Caution: Very corrosive. Generates heat on dilution. Where stock sulfuric acid is 18 M, add 13.9 ml to 86 ml H₂O. Store at RT.

EIA-grade microplates (Immulon4-HBX), microplate washer, and microplate reader.

ASSAY PROCEDURE

Coating of plates:

Dilute the capture antibody 1/100 in coating buffer (preferably in a polypropylene tube) and immediately add 100 µl to every well in the microplate. Incubate overnight at 4°C.

Blocking:

Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for a minimum of 90 minutes at 22°C. Wash plate three times with wash buffer.

Samples:

Reference plasma is diluted 1/200 (100%) then serial 1/2's down to 1/6400 (3.13%). Sample plasmas are diluted 1/400, 1/800 and 1/1600. All dilutions are made in HBS-BSA-Tween 20 sample diluent. Apply 100 µl/well and incubate plate at 22°C for 90 minutes. Wash plate three times with wash buffer.

Detecting Antibody:

Dilute the detection antibody 1/100 in HBS-BSA-Tween 20 sample diluent and apply 100 µl to each well. Incubate plate at 22°C

for 90 minutes. Wash plate three times with wash buffer.

OPD Substrate:

Apply 100 µl of freshly prepared OPD substrate to every well. Allow color to develop for 10-15 minutes then stop color reaction with the addition of 50 µl/well of 2.5 M H₂SO₄. The plate can be read at a wavelength of 490 nm.

TECHNICAL NOTES

- This paired antibody product is intended to facilitate the end user in establishing an in-house immunoassay for research purposes only. It must not be used for diagnostic applications. Assay validation is the responsibility of the end user and should be done according to user-defined protocols
- Reference calibrators should be of the same matrix and anticoagulant as the samples to be tested (example serum or plasma, citrate or EDTA).
- Do not use samples diluted less than 1/10 as falsely high readings may result.
- The optimal color development time should be determined empirically as the time required to obtain an absorbance of at least 1.000 at 490 nm for the 100% reference point, not to exceed 20 minutes.
- Rheumatoid factor in samples may interfere in ELISA by binding to the capture and/or detecting antibodies. The wells should not be allowed to become dry. Keep plate covered or in a humid chamber during incubations.
- Antibodies are supplied in a 50% glycerol solution and can be centrifuged briefly in a micro-centrifuge to gather residual reagent from the cap and walls of the tube.