Creative Diagnostics®

Version 01-04/25

User's Manual

Canine Factor VIII Matched Antibody Pair

REF **ABPR-L008**

Σ **5 x 96 tests**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This antibody pair set comes with matched antibody pair to detect and quantify protein level of Canine Factor VII

General Description

Factor VIII is a large glycoprotein (320 kDa) synthesized in the liver. The majority of Factor VIII is cleaved during expression, resulting in a mixture of partially cleaved forms ranging in size from 200-280 kDa. The F.VIII is stabilized in circulation through non-covalent association with von Willebrand Factor. The concentration of F.VIII in normal human plasma is typically 200 ng/mL. In canine plasma, the F.VIII activity is 5-7 fold higher relative to human plasma. F.VIII is a pro-cofactor that is activated through limited proteolysis by thrombin. In this process F.VIIIa dissociates from vWF to combine with activated Factor IX, calcium and a phospholipid surface where it is an essential cofactor in the assembly of the Factor X activator complex. Hemophilia A is a congenital bleeding disorder resulting from an X-chromosome-linked deficiency of F.VIII, occurring with a frequency of 1 in 4000 males. The defect can be caused by any one of hundreds of reported mutations but are most commonly due to inversions within intron 22 of the F.VIII gene. Hemophilia A has also been reported in a variety of species including dog and mouse, with a clinical phenotype very similar to human. The genetic defect in one case of canine Hemophilia-A has been shown to also be due to a gene inversion similar to the human defect, possibly indicating a common instability of the F.VIII gene in humans and dogs.

Principles of Testing

Purified antibody to canine FVIII is coated onto the wells of a microtitre plate. The plate is washed and plasma or other fluids containing cFVIII are applied. The coated antibody will capture the cFVIII in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to cFVIII is added to the plate to bind to the captured cFVIII. 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 colour produced is quantified using a microplate reader. The colour generated is proportional to the concentration of canine FVIII present in the sample.

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.4 ml of polyclonal purified anti-canine FVIII antibody for coating plates.
2. Detecting Antibody (neutral): Four neutral-capped tubes each containing 10 ml of pre-diluted peroxidase conjugated polyclonal anti-canine FVIII antibody for detection of captured cFVIII.
3. Sample Diluent (green): 100 ml bottle containing a green-coloured diluent optimised for dilution of samples.

Note: Reagents are sufficient for at least 4×96 well plates using recommended protocols.

Materials Required But Not Supplied

1. Coating Buffer: 50 mM Carbonate
2. PBS:(base for wash buffer)
3. Wash Buffer: PBS-Tween(0.1%, v/v)
4. Substrate Buffer: Citrate-Phosphate buffer pH 5.0
5. OPD Substrate:(o-Phenylenediamine. 2HCl) TOXIC!
6. Stopping Solution: 2.5 M H2SO4
7. Other: Microplates; Microplate washer; Microplate reader.

Storage

2-8°C

Assay Procedure

1. **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 plate. Incubate 2 hours at 22°C or overnight at 2-8°C.
2. **Blocking:** Blocking is not required under the conditions described. Washing the plate with PBS-Tween is sufficient to block non specific interactions. Wash plate 3 times with wash buffer.
3. **Samples:** To prepare a reference curve normal canine plasma is diluted 1/5(100%) then serial 1/2's down to 1/160(3.13%). Sample plasmas are diluted 1/10, 1/20 and 1/40. All dilutions are made in the provided green sample diluent. Apply 100 µl/well and incubate plate at 22°C for 120 minutes. Wash plate 3 times with wash buffer.
4. **Detecting Antibody:** Apply the pre-diluted detecting antibody, 100 µl to each well. Incubate plate at 22°C for 60 minutes. Wash plate 3 times with wash buffer.
5. **OPD Substrate:** Apply 100 µl of freshly prepared OPD substrate to every well. Allow colour to develop for 5-10 minutes then stop colour reaction with the addition of 50 µl/well of 2.5 M H2SO4. The plate can be read at wavelength of 490 nm.