REAADS Collagen Binding Assay Test Kit

Product #: 11160
(96 well kit)

Not FDA Cleared for Diagnostic Use in the United States. *Performance characteristics have not been established.*

- **Reagent complete kit, convenient procedure**
- **96-well microplate format**
- **2.5 hour total incubation at room temperature**
- **May be used in conjunction with vWF:Ag assays to differentiate between vWD Types I and II**

Background

Von Willebrand Factor (vWF) is an important blood clotting protein, involved in both assisting platelet adhesion and stabilization of clotting factor VIII. In von Willebrand Disease (vWD), there is either a partial quantitative deficiency, i.e. shortage of vWF (classified as vWD Type I), or a qualitative, i.e. functional deficiency (classified as vWD Type II). VWD Type III is rare and characterized by virtually complete deficiency of vWF. Higher molecular weight multimers of von Willebrand factor (vWF) serve to bind activated platelets through specific membrane glycoproteins to connective tissue fibers exposed at wound sites and thus promote blood clotting and wound healing.

The worldwide incidence of vWD is estimated at 1% to 3% but may be more common since mild cases may remain undetected. The Collagen Binding Assay (CBA) is an ELISA procedure that quantitates the binding of vWF to collagen type III coated onto microtiter wells. Collagen binding of vWF is associated with the higher molecular weight (HMW) forms of vWF, believed to be functionally more important in hemostasis than lower molecular weight (LMW) forms. Therefore CBA may correlate more closely with vWF function and bleeding problems than other ELISAs for vWF which measure total (LMW + HMW) vWF.

Test Principle

During the first incubation step the von Willebrand Factor multimers present in the sample bind to the collagen which is attached to the surface of the microtiter plate. Unbound plasma proteins are then removed by washing and in a second reaction, peroxidase conjugated antibodies are bound to anti-human vWF multimers. Excess antibody is washed off and the bound enzyme activity is determined by the addition of TMB substrate. The resulting color intensity, which is proportional to high molecular weight vWF multimers present in the sample, is determined photometrically. The supplied calibrated standards may be used to assist in quantifying the activity of the high molecular weight vWF multimers.

Test Procedure

Von Willebrand Disease (vWD) is characterized by an abnormal function or biosynthesis of the von Willebrand factor (vWF). The complete diagnostic classification of von Willebrand Disease requires both functional and antigenic assays. Functional assays measure qualitative deficiencies while antigenic assays measure quantitative abnormalities of vWF molecule.

Procedure

1. Remove required number of microwell strips from foil pouch.
2. Add 100ul of diluted standards and controls to duplicate wells.
3. Add 100ul of diluted samples to duplicate wells.
4. Add 100ul of working buffer solution to duplicate wells for use as a zero point on the standard curve.
5. Ensure that all samples are added within 5 minutes to minimize variation in incubation times. Mix by tapping gently on all 4 sides or using a mechanical mixer.
6. Cover plate (or place in moist chamber) and incubate for 60 minutes at room temperature.
7. Reconstitute conjugate at least 5 minutes prior to use.
8. Thoroughly aspirate contents of all wells.
9. Wash plate 3 times by filling all wells with 300ul of working buffer solution, then aspirating. Tap upside down on blotting paper after final aspiration.
10. Add 100ul of conjugate to each well. Mix by tapping gently on all 4 sides or using a mechanical mixer.
11. Cover (or place in moist chamber) and incubate for 60 minutes at room temperature.
12. Wash plate 4 times (see step 9).
13. Add 100ul/well of TMB substrate solution.
14. Incubate uncovered for 5 minutes at room temperature.
15. Add 100ul stop solution to each well, adding at the same rate and in the same sequence as the substrate.
16. Within 30 minutes, read the absorbance at 450nm, or at 450nm with a 650± 50nm reference if dual wavelength plate reader available.

Normal Range:	50-400%
Intra-assay Precision:	3.9%
Kit Shelf Life:	12 months

Rev. 2003-02-26
900016

Corgenix, Inc.
12061 Tejon Street
Westminster, Colorado 80234
Toll Free: 800-729-5661
Telephone: 303-457-4345
Fax: 303-457-4519
Email: techsupport@corgenix.com
www.corgenixonline.com

Corgenix (UK) Ltd.
75 Broadway
Peterborough, PE1 1SY UK
Telephone: +44 (0) 1733 296800
Fax: +44 (0) 1733 296809
Email: sales@corgenix.co.uk
www.corgenix.co.uk

Corgenix Medical Corporation trades on the NASD (OTC BB) under the ticker symbol CONX. Corgenix, Inc. operates under FDA Quality System Regulations and is ISO 9001:1994/ISO 13485/EN 46001 certified.