REAGENTS

A. Description of Reagent Items

Item 1: Foil pouch containing 8 strips, each containing 16 wells coated with sheep antibody to human FVIII.

Item 2: 2 vials of Calibrator Plasma, each lyophilized from 1 mL plasma.

Item 3: 2 vials of Control Plasma A, each lyophilized from 1 mL plasma.

Item 4: 2 vials of Control Plasma B, each lyophilized from 1 mL plasma.

Item 5: 1 vial containing 50 mL of 20X Wash Buffer Concentrate.

Item 6: 3 vials, each containing 20 mL of buffered Sample Diluent.

Item 7: 1 vial containing 12 mL peroxidase-labeled sheep detecting antibody.

Item 8: 1 vial containing 12 mL of tetramethylbenzidine (TMB) substrate.

Item 9: 1 vial containing 12 mL Stop Solution (0.2 M Sulphuric acid).

B. Caution and Warning

This kit is intended for use by personnel trained in laboratory procedures and universal precautions for the use of chemicals and potentially biohazardous substances. Some items contain human source material. Each unit of source plasma used in the preparation of this product has been tested by FDA approved methods and found negative for HBsAg, syphilis and antibodies to HIV and HCV and non-reactive for HIV-1 nRNA and HCV nRNA. However, no test can offer complete assurance that products derived from human blood will not transmit infectious diseases. As with all materials of human origin, this product should be handled as a potentially infectious material.

The substrate TMB (tetramethylbenzidine) has reduced toxicity, but precautions should still be taken to avoid direct contact. The use of gloves and safety glasses is recommended. The Stop Solution contains dilute sulphuric acid (0.2 M), which is corrosive. The use of gloves and safety glasses is recommended. The disposal of waste materials must be carried out according to current local regulations. For a Material Safety Data Sheet for this product contact Affinity Biologicals Inc.

C. Reagent Preparation

Item 1 (Antibody-coated strips with frame): Just prior to use, open pouch and remove strips and frame. Unused strips should be replaced in the pouch and resealed. Strips may be used directly, see Procedure section C: Assay Procedure.

Item 2 (Calibrator plasma): Reconstitute one vial with 1.0 mL of reagent grade water. Allow contents to dissolve for 15 minutes at room temperature with occasional swirling. Stability after reconstitution is 4 hours at ambient (18-25°C), or 30 days at –20°C.

Item 3 and 4 (Control plasmas): Reconstitute one vial of each plasma with 1.0 mL of reagent grade water. Allow contents to dissolve for 15 minutes at room temperature with occasional swirling. Stability after reconstitution is 4 hours at ambient (18-25°C), or 30 days at –20°C.

Item 5 (20X Wash Buffer Concentrate): Allow vial to warm to room temperature before use. Ensure any crystals that may have formed are dissolved before proceeding. If necessary the vial can be warmed to 37°C until all crystals have dissolved. Dilute the concentrate 1/20 before use. For every 2 strips (32 wells), add 16 mL concentrate to 304 mL reagent grade water and mix. Stability after dilution is 1 week at 2–8°C.

Items 6-9 are supplied ready to use.

D. Storage and Stability

Intact kits and un-reconstituted reagents are stable until the expiration date stated on the box and individual reagent labels when stored at 2–8°C.

SPECIMEN COLLECTION

Blood is collected into 3.2% Buffered Citrate anticoagulant tubes at a ratio of 9 volumes blood to 1 volume anticoagulant and gently mixed by inversion. Centrifuge at a minimum of 1500 x g for 15 minutes (CLSI Guideline H21-A5). Remove supernatant plasma and use within 4 hours or freeze below –20°C for up to 1 month.

PROCEDURE

A. Material Provided

Foil pouch containing 6 strips of antibody coated wells.

Calibrator Plasma, lyophilized.

Control Plasma A, lyophilized.

Control Plasma B, lyophilized.

20X Wash Buffer Concentrate.

Sample Diluent.

Detecting antibody solution.

TMB substrate.

Stop Solution.

Adhesive Plate Sealer.
C. Assay Procedure

**PROCEDURAL NOTES:**

- Reconstitute reagents as described in REAGENTS, Section C, Reagent Preparation. Allow reagents to warm to room temperature before use.
- It is recommended that all calibrator, control and test sample dilutions be run in duplicate and that each run include a buffer blank (see Assay Calibration section).
- All dilutions must be made just prior to use in the assay.
- Do not allow the wells to become dry at any time. Keep plate covered during incubations.
- Plasma samples should not be applied at dilutions lower than 1/4.
- Do not use kit components from different lot numbers.
- Incubation temperatures above or below normal room temperature (18 - 25°C) may contribute to inaccurate results.
- Do not use kit components beyond expiration date.
- Do not allow the wells to become dry at any time. Keep plate covered during incubations.
- It is recommended that all calibrator, control and test sample dilutions be run in duplicate and that each run include a buffer blank (see Assay Calibration section).
- All dilutions must be made just prior to use in the assay.
- Do not allow the wells to become dry at any time. Keep plate covered during incubations.
- Plasma samples should not be applied at dilutions lower than 1/4.
- Do not use kit components from different lot numbers.
- Incubation temperatures above or below normal room temperature (18 - 25°C) may contribute to inaccurate results.
- Do not use kit components beyond expiration date.
- Do not allow the wells to become dry at any time. Keep plate covered during incubations.

1. **Preparation of Calibrator Plasma Dilutions:** Dilute the Calibrator Plasma (reconstituted Item 2) into Sample diluent (Item 6) as detailed in Table 1 below:

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Calibrator Plasma</th>
<th>Sample Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% **</td>
<td>175 µL</td>
<td>525 µL</td>
</tr>
<tr>
<td>50%</td>
<td>350 µL of 100%</td>
<td>350 µL</td>
</tr>
<tr>
<td>25%</td>
<td>350 µL of 50%</td>
<td>350 µL</td>
</tr>
<tr>
<td>12.5%</td>
<td>350 µL of 25%</td>
<td>350 µL</td>
</tr>
<tr>
<td>6.25%</td>
<td>350 µL of 12.5%</td>
<td>350 µL</td>
</tr>
<tr>
<td>3.13%</td>
<td>350 µL of 6.25%</td>
<td>350 µL</td>
</tr>
<tr>
<td>1.56%</td>
<td>350 µL of 3.13%</td>
<td>350 µL</td>
</tr>
<tr>
<td>0.78%</td>
<td>350 µL of 1.56%</td>
<td>350 µL</td>
</tr>
</tbody>
</table>

**NOTE:** 100% = 1.0 IU/mL

**Refers to Calibrator plasma vial (Item 2) for FVIII antigen value to be used as the concentration of the initial dilution of the calibrator plasma. E.g. If the calibrator has an assigned value of 1.25 IU/mL follow the dilution scheme above but call the first point of the calibration curve 1.25 IU/mL.

2. **Control plasma A (reconstituted Item 3) and normal test plasmas** are diluted 1/8 and 1/16. Add 100 µL plasma to 700 µL sample diluent (Item 6), mix, then add 350 µL of this 1/8 dilution into 350 µL sample diluent to obtain the 1/16 dilution. **Control Plasma B** (reconstituted Item 4) and samples low in FVIII antigen (Haemophilic samples) should be run at lower dilutions of 1/4 and 1/8. Add 175 µL plasma into 525 µL sample diluent (Item 6), mix, then add 350 µL of this 1/4 dilution into 350 µL sample diluent to obtain the 1/8 dilution.

3. **Assay**

**PLATE PREPARATION:** Place desired number of strips into frame.

**STEP** Pipette into each pre-coated well:

**FVIII CAPTURE**

<table>
<thead>
<tr>
<th>Test Sample (run in duplicate)</th>
<th>100 µL</th>
</tr>
</thead>
</table>

Cover strips with the plate sealer and incubate 1 hour at ambient temperature.

**DETECTING ANTIBODY**

<table>
<thead>
<tr>
<th>Detecting Antibody Solution (Item 7)</th>
<th>100 µL</th>
</tr>
</thead>
</table>

Cover strips with the plate sealer and incubate 45 minutes at ambient temperature.

**COLOR**

<table>
<thead>
<tr>
<th>1MB Substrate (Item 8)</th>
<th>100 µL</th>
</tr>
</thead>
</table>

**EXPECTED VALUES**

The normal range for Factor VIII as reported in the literature is 0.5-1.8 IU/mL. Each laboratory should determine a normal range independently but results from three lots measured in 99 healthy individuals indicate a normal reference interval for FVIII antigen of 0.64-1.89 IU/mL (mean 1.268 IU/mL, SD = 0.3116).

**PERFORMANCE CHARACTERISTICS**

A. **Specificity**

This assay measures Factor VIII antigen in human plasma, therapeutic Factor VIII concentrates and recombinant Factor VIII preparations.

B. **Detection Limit**

When assay is performed as indicated in Section C, Assay Procedure, the detection limit of this assay is 0.008 IU/mL (0.8 %) Factor VIII antigen. The upper limit of detection may vary with each lot of kit depending on the assayed value of the calibrator plasma supplied in the kit. Samples with
values outside the range of the reference curve should be re-tested at an appropriate dilution to obtain accurate results.

C. Method Comparison:
The average results of three lots of the VisuLize™ Factor VIII Antigen kit were compared internally to the Coamatic® FVIII Assay on 142 patient samples containing Factor VIII levels ranging across the entire detection range. The correlation co-efficient (r) was 0.970 (R\(^2\) = 0.940, y = 1.2059x + 0.0535). The VisuLize™ Factor VIII Antigen kit was also compared at two external testing sites to the Coamatic® FVIII Assay on patient samples with Factor VIII levels ranging across the entire detection range. At external site #1, 110 samples were tested and the correlation co-efficient (r) was 0.996 (R\(^2\) = 0.9931 y = 1.2261x + 0.1085). At external site #2, 81 samples were tested and the correlation co-efficient (r) was 0.974 (R\(^2\) = 0.9488, y = 1.1768x + 0.0242).

D. Precision
Intra-assay Precision, Method 1: In each of three lots of product, normal and abnormal plasma samples were tested in 4 assays total with 38 replicates per sample per plate. The mean coefficient of variation (CV) from all results was 4.56%.

Intra-assay Precision, Method 2: Three plasmas with different Factor VIII antigen concentrations were tested in replicates of 8 in 20 assay events using 3 lots of product. The intra-assay coefficient of variation (CV) was calculated according to NCCLS Guideline EP5-A\(^3\) and is indicated in the summary below for each Factor VIII level. The mean CV from all results by this method was 4.51%.

Inter-assay Precision: Three plasmas with different Factor VIII antigen concentrations were tested in replicates of 8 in 20 assay events using 3 lots of product. The inter-assay coefficient of variation (CV) was calculated according to NCCLS Guideline EP5-A\(^3\) and is indicated in the summary below for each Factor VIII level. The mean CV from all results by this method was 4.69%.

E. Lot-to-Lot Variability
94 control samples with Factor VIII antigen values ranging from 0.23-2.3 IU/mL were tested in duplicate on three lots to determine assay precision between lots. The mean lot-to-lot variability was 7.78%.

REFERENCES