**Supplied Materials:**

1. **Capture Antibody (VWF-EIA-C):** One yellow-capped vial containing 0.5 ml of polyclonal purified anti-vWF antibody for coating plates.

2. **Detecting Antibody (VWF-EIA-D):** One red-capped vial containing 0.5 ml of peroxidase conjugated polyclonal antibody for detection of captured vWF.

**Note:** Antibodies are supplied in a 50% (v/v) glycerol solution for storage at -10 to -20°C. Keep vials tightly capped. Do not store in frost-free freezers.

**Materials Required but not Provided:**

1. **Coating Buffer:** 50 mM Carbonate 1.59g of Na2CO3 and 2.93g of NaHCO3 up to 1 litre. Adjust pH to 9.6. Store at 2-8°C up to 1 month.

2. **PBS:** (base for wash buffer and blocking buffer) 8.0g NaCl, 1.15g Na2HPO4, 0.2g KH2PO4 and 0.2g KCl, up to 1 litre. Adjust pH to 7.4, if necessary. Store up to 1 month at 2-8°C, discard if there is evidence of microbial growth.

3. **Wash Buffer:** PBS-Tween (0.1%, v/v) To 1 litre of PBS add 1.0 ml of Tween-20. Check that the pH is 7.4. Store at 2-8°C up to 1 week.

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

5. **Sample Diluent:** PBS-BSA-T20 5.95g HEPES (free acid), 1.46 g NaCl, 2.5 g Bovine Serum Albumin (Sigma, RIA grade) dissolved in 200 ml of PBS. Add 0.25 ml of Tween-20, check and adjust pH to 7.2 with NaOH, then make up to a final volume of 250 ml with H2O. Aliquot and store frozen at -20°C.

6. **Substrate Buffer:** Citrate-Phosphate buffer pH 5.0 2.6g Citric acid and 6.9g Na2HPO4 up to a final volume of 500 ml with purified H2O. Store at 2-8°C up to 1 month.

7. **OPD Substrate:** (o-Phenylenediamine,2HCl) Toxic! (5mg tablets: Sigma # P-6912). Make up immediately before use. Dissolve 5mg OPD in 12 ml substrate buffer then add 12 µl 30% H2O2. Do not store.

8. **Stopping Solution:** 2.5 M H2SO4 Caution: VERY CORROSIVE! GENERATES HEAT ON DILUTION! Where stock sulphuric acid is 18 Molar, add 13.9 ml to 86 ml H2O. Store at room temperature.

9. **Other:**
   - Microplates, 96-well Immulon 4-HBX (http://www.labsystems.fi)
   - Microplate washer (optional)
   - Microplate reader.
### 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 hrs @ 22°C or overnight at 2-8°C.

2. **Blocking:**
   Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for 60 minutes @ 22°C. Wash plate X 3 with wash buffer.

3. **Samples:**
   Reference plasma is diluted 1/100 (100%) then serial 1/2's down to 1/3200 (3.13%). Sample plasmas are diluted 1/200, 1/400 & 1/800. All dilutions are made in HBS-BSA-T20 sample diluent. Apply 100 µl/well and incubate plate @ 22°C for 90 minutes. Wash plate X 3 with wash buffer.

4. **Detecting Antibody:**
   Dilute the detecting antibody 1/100 in HBS-BSA-T20 sample diluent and apply 100 µl to each well. Incubate plate @ 22°C for 90 minutes. Wash plate X 3 with wash buffer.

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

### Calculation of Results:

The construction of a proper reference curve is of no less importance than any other aspect of the assay. A reference curve should be constructed by plotting the known concentration of standards versus absorbance. This can be done manually using graph paper, or by using curve-fitting computer software. In our experience, the dose response curves of most immunoassays tend to be sigmoid in shape. Although linear regions can be identified within the curve, the best overall fit is of ten obtained using an algorithm that provides a weighted theoretical model of fit throughout the entire curve, such as a 4-parameter or 5-parameter logistic curve fit 4,5. In general, the simplest model that defines the concentration-response relationship should be used 6. The “back-fit” test is a simple and reliable method to determine if a curve-fitting method is appropriate. In this test, the apparent correlation coefficient (R2) is a valuable indicator of the overall fit, but should not be used by itself in the selection of a curve fitting method, as a poor fit in a particular region of the curve may not be evident from this value alone 5,6.

In the quality control of this product we have determined that under the conditions described above, a reference curve that is constructed using serial dilutions of normal pooled plasma, will produce a correlation coefficient (R2) of at least 0.980 using a log-log fit, and an R2 of at least 0.990 using a 4-parameter logistic curve fit algorithm. However, the performance characteristics of in-house assays developed using this product in other laboratories may vary slightly from ours. Different curve fitting methods may be employed but we recommend that the back-fit test be applied as evidence that the fitting method is appropriate.

### 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 6.
- 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 colour 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.

### References:

2. Sadler JE; von Willebrand Factor (Minireview); JBC 266:34, pp 22777-22780, 1991.