ELISA Protocol: Quantitative Measurement of VWF Propeptide in Plasma
Catalog Number: CS-MW1939 Anti-human VWF Propeptide Antibody Pair

Antibody Pair Components:

Quantity	Component	Presentation	Volume
1 vial	CS-MW1939-A Coating Antibody CLB-Pro 35	100-fold concentrated	375 µl
1 vial	CS-MW1939-B HRP-conjugated Antibody CLB-Pro 14.3	100-fold concentrated	375 µl

Keep all reagents in frozen aliquots at -18ºC. Avoid repeated freeze-thaw cycles.

Additional Materials Required:

Plasma Standard
A pool of normal plasma from healthy donors can be used as standard. Blood should be collected in vacutainer tubes containing 3.2% buffered citrate solution (1:9 vol/vol) and immediately placed on ice and centrifuged at 3000 g for 20 minutes at 2 – 8ºC. Keep plasma frozen in working aliquots.

Microtiter Plates
We recommend Nunc MaxiSorp® flat-bottom 96 well plates, or similar.

Coating Buffer
0.1 M carbonate/bicarbonate buffer, pH 9.6

Dilution Buffer
Phosphate Buffered Saline / 0.1% Tween-20 / 1% BSA
Tween-20 (Merck 822184)
BSA (Sigma A-7030)

Wash Buffer
Phosphate Buffered Saline / 0.1% Tween-20

Substrate, Buffer and Stop Solutions for enzymatic color development
Recommended: TMB (3,3',5,5'-Tetramethyl-benzidine; Sigma, T2885)
Substrate Buffer: 0.11 M acetate buffer, pH 5.5
TMB Stock Solution: 6 mg/ml TMB in DMSO
Hydrogen Peroxide Stock Solution: 3% H_2O_2 solution in distilled water

Substrate Solution:
For each plate mix the following reagents:
12 ml Substrate Buffer
200 µl TMB Stock Solution
12 µl H_2O_2 Stock Solution

Stop Solution:
1.8 M H_2SO_4 solution in distilled water

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ELISA Method

Note: When testing plasma samples, citrated plasma should be used.

Day 1

Depending on the number of samples to be tested, one or more plates should be coated the day before the actual test.

Coating:
The coating antibody CS-MW1939-A is provided as a 100-fold concentrated solution. The vial contains enough to coat three 96 well plates, including the standard curve samples.

For each 96 well microtiter plate,
Add 120 µl coating antibody to 12 ml coating buffer.
Add 100 µl to all wells, cover microtiter plate with adhesive seal and incubate overnight at 4°C.

Day 2

1. **Blocking Step**
   Wash the plate 5 times with wash buffer.
   Aspirate supernatants from wells and completely fill wells (>300 µl) with wash buffer.
   Repeat this four times. After the final aspiration, the wells should be dry.

   Add 200 µl Dilution Buffer to all wells.
   Cover plate with adhesive seal and incubate for 2 hours at 37°C.

2. **Standard Curve and Samples**
   A standard curve is prepared by a 2-fold serial dilution starting with a 1:10 dilution of the plasma standard in Dilution Buffer.

   Example:
   Label 8 tubes, one tube for each standard dilution 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280.
   Pipette 270 µl of dilution buffer into the tube labelled 1:10 and 150 µl of dilution buffer into the other tubes.
   Transfer 30 µl of the plasma standard into the first tube labelled 1:10. Mix well, and transfer 150 µl of this dilution into the second tube (1:20).
   Repeat the serial dilutions six more times by adding 150 µl of the previous tube of diluted plasma standard to the 150 µl of dilution buffer.
   It is recommended to prepare two separate standard curves for each assay.

   For individual samples (citrate-plasma) 1:40, 1:80, 1:160, 1:320 dilutions in duplicate should be made.

   Wash the microtiter plate 5 times with wash buffer as described above.

   Transfer 100 µl of the prepared standards and samples into the appropriate wells. Dilution buffer can be used for blanks.

   Cover plate with adhesive seal. Incubate for 2 hours at 37°C.

3. Incubation with HRP-conjugated Antibody

Just before washing, prepare next incubation reagent.

HRP-conjugated antibody CS-MW1939-B, is provided as a 100-fold concentrated solution. The vial contains enough for three 96 well plates, including the standard curve samples.

For each 96 well microtiter plate,
Add 120 µl HRP-conjugated antibody to 12 ml dilution buffer.
Add 100 µl to each well.
Cover microtiter plate with adhesive seal and incubate for 2 hours at 37°C.

4. Incubation with TMB Substrate

Wash the microtiter plate 5 times with wash buffer as described in step 1.
Add 100 µl of the TMB-substrate solution to all wells.
Gently agitate by tapping the edge of the microtiter plate for a few seconds to mix the contents of each well.
Incubate in a dark place until a visual difference in color is noticeable between the 1:320 and 1:640 dilutions of the standard curve (approximately 15 minutes).
Stop the enzymatic reaction by adding 100 µl of Stop Solution to all wells.
Read absorbance at 450 nm *within 30 minutes* in an ELISA reader.

Note: The speed of enzymatic color development is influenced by many factors, including temperature and quality of the TMB used.

5. Results and Interpretation of Data

- Record the absorbance at 450 nm for each well and average the duplicate values.
- For each sample, duplicates should not differ more than 15% from the mean value. If duplicates vary more, the assay should be repeated.
- Plot the average absorbance of the calibrators (y-axis, linear scale) versus the related plasma standard-dilution in percentage (x-axis, logarithmic scale) and draw the best fitting curve employing a suitable curve fitting computer program.
- Expected levels of VWF propeptide in fresh plasma samples of healthy individuals lie between 60 and 140% of plasma standard made from healthy donors.
- Interpolate the average absorbance values for each sample on the reference curve.
- Test samples which show a mean absorbance outside the range of the reference curve dilutions should be diluted appropriately.