vonWillebrand Factor (vWF) Antigen Assay

vonWillebrand Factor antigen is detected by binding to an antibody, then secondary binding by a conjugated antibody that will produce a detectable color change with the addition of a substrate

Materials

1. Versa Max microtiter plate reader (Molecular Devices, CA)
2. 96-well microtiter plate
3. Citrated plasma samples
4. Normal mouse plasma (NMP)
5. 1 µg/µL rabbit anti-human vWF polyclonal antibody in 50 mM Na_2CO_3, pH 9.6
6. 3% BSA in TBS
7. 1% BSA in TBS
8. 0.05% Tween 20 in TBS
9. Horseradish peroxidase (HRP)-conjugated rabbit anti-human vWF polyclonal antibody diluted 1:2000 in 1% BSA in TBS
10. 1 N H_2SO_4

Procedure

1. Plasma samples may be diluted to 1:200 in TBS/1% BSA
2. Log-log standard curve is prepared with each plate by diluting NMP 1:25 to 1:1600 in TBS/1% BSA, analyzed simultaneously on the sample plate
3. Plate wells are loaded as follows:

Block	**100uL**	**Rabbit anti-human vWF Ab**	**5℃**	**Overnight**
Block	**200uL**	**3% BSA in TBS**	**5℃ or 37℃**	**Overnight Or 3-5hr**
Wash 1x		**1% BSA in TBS**		
	100uL	**Sample dilutions, in duplicate**	**5℃**	**Overnight**
Wash 5x		**TBS/Tween**		
	100uL	**HRP-conjugated rabbit anti-human vWF Ab**	**5℃**	**Overnight**
Wash 5x		**TBS/Tween**		
Color development	**100uL**	**TMB peroxidase substrate**		
Stop reaction	**100uL**	**1N H2SO4**		

4. Plate is read at 655 nm during development and then at 450 nm after reaction is stopped
5. Absorbance are converted to %NMP vWF using the standard curve