Protein S Assay

Protein S 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. 10 µg/µL rabbit anti-human Protein S polyclonal antibody in 50 mM Na_2CO_3, pH 9.6
6. TBS
7. 3% BSA in TBS
8. 1% BSA in TBS
9. 0.05% Tween 20 in TBS
10. Horseradish peroxidase (HRP)-conjugated rabbit anti-human Protein S polyclonal antibody diluted 1:1000 in 1% BSA in TBS
11. 1 N H_2SO_4

Procedure

1. Plasma samples may be diluted to 1:100 - 1:200 in TBS/1% BSA
2. Log-log standard curve is prepared with each plate by diluting NMP 1:25 to 1:100 in TBS/1% BSA, analyzed simultaneously on the sample plate
3. Plate wells are loaded as follows:

Rabbit anti-human protein S Ab	**100uL**	**5℃**	**Overnight**	
3%BSA in TBS	**200uL**	**37℃ or 5℃**	**3-5hr or Overnight**	**Block**
1%BSA in TBS				**Wash 1x**
Sample dilutions, in duplicate	**100uL**	**5℃**	**Overnight**	
0.05% Tween 20 in TBS				**Wash 5x**
Diluted HRP-conjugated rabbit Ab in TBS/1%BSA	**100uL**	**5℃**	**Overnight**	
0.05% Tween 20 in TBS				**Wash 5x**
TMB peroxidase substrate			**4hr**	**Color development**
H2SO4	**100uL**			**Stop reaction**

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