Protein C Assay

Measured by the level of chromogenic substrate cleaved by activated protein C

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. TBS with 100 mM CsCl
6. 2 U/mL Protein C activator
7. 2.5 mM chromogenic substrate specific for activated Protein C (APC)
8. 20% acetic acid

Procedure

1. Plasma samples may be diluted to 1:10 - 1:20 in TBS-CsCl
2. Standard curve is prepared with each plate by diluting NMP 1:4 to 1:64 in TBS, analyzed simultaneously on the sample plate
3. Plate wells are loaded as follows:

Sample dilutions, in duplicate	**10uL**	**37℃**	**15min**	
Protein C activator	**25uL**	**37℃**	**15min**	
Chromogenic substrate	**25uL**	**37℃**	**1hr**	**Color development; plate must be covered**
Acetic acid	**25uL**	**37℃**	**1hr**	**Stop reaction**

4. Plate is read at 405 nm
5. Absorbance are converted to %NMP Protein C using the standard curve