Determination of Activated Protein C (APC)

For Research use only. Not for use in diagnostic procedures.

Reagents:

1) Buffer A: Tris 0.05 M, CsCl 0.26 M, $CaCl_2$ 0.004 M, pH 8.30
2) Buffer TBSA (Ref AR005): Tris 0.05 M, NaCl 1.5 M, BSA 1%, pH 7.40
3) Chromogenic Substrate CS-21(66), 25mg (Ref 229021): Reconstitute one vial with 10 mL aqua dest
4) Calibrator: Human APC, 10, 100 or 1000 µg (Ref: EZ004 or HAPC): Reconstitute with TBSA buffer to a stock solution of 2.5 µg/mL, make further dilutions with TBSA buffer.

Manual Method:

Into a microwell or a plastic test tube, incubated **at 37°C**, introduce:

Reagent	Microplate	Test Tube
Buffer A	100 µL	400 µL
Sample, Calibrator or Control	25 µL	100 µL
Mix and incubate for **1** min at 37°C, then introduce:		
Chromogenic Substrate preincubated at 37°C	25 µL	100 µL
Mix and incubate for **5** min at 37°C, **exactly**		
Stop the reaction by introducing:		
Citric Acid 2%, or acetic Acid 20%	75 µL	300 µL
Mix and measure the Absorbance at **405nm** against a sample blank.		