

#### Intended Use

Diagen Activated Protein C (APC) Resistant Plasma may be used as a positive control for reagent systems used for the detection of the factor V Leiden mutation (FV:Q<sup>506</sup>).

#### Summary and Principle

Activated Protein C (APC) is a serine protease, an important anticoagulant enzyme that is required for the regulation of blood coagulation. APC acts by inactivating pro-coagulant factors Va and VIIIa. Normally, factor Va is inactivated by an initial cleavage of the peptide bond on the carboxyl side of arginine 506 followed by a second cleavage at arginine 306. In patients with the (FV:Q<sup>506</sup>) mutation, the 506 cleavage is inhibited causing a much slower cleavage rate leading to the phenomenon of resistance to the anticoagulant activity of APC.

#### Reagent

**APC Resistant Control Plasma** **10 vials**  
Lyophilised, buffered plasma, collected from donors heterozygous to the Factor V Leiden mutation. For reconstitution, remove the cap and rubber stopper and add 0.5 mL of distilled water. Allow 5 to 10 minutes for complete solution.

#### Warnings and Precautions

##### POTENTIAL BIOHAZARD MATERIAL.

Diagen APC Resistant Control Plasma is of human origin. All donor units used in production of this product have been found negative for anti HIV, anti HCV, HBsAg and Syphilis by approved methods. However, all plasma of human origin should be considered as potentially infectious and handled appropriately. Please refer to the relevant SDS Sheet for handling and safety procedures. Dispose of all waste materials according to the stated international, national and local regulations.

#### Collection of Blood Samples

Blood (9 parts) is collected into 1 part of 3.2% trisodium citrate and the plasma obtained by centrifugation at 2500 g for 15 minutes. The plasma should be stored in stoppered tubes. The use of 3.2% citrate containing 5% HEPES buffer improves the stability of both fresh and deep frozen plasma.

#### Procedure

##### Materials Provided

Materials needed for Activated Protein C resistance screening are shown below:

##### Cat. No.

APCC400 – APC Resistant Control Plasma.

##### Materials and equipment required, but not provided:

1. General routine laboratory coagulation equipment.
2. Reaction cups or test tubes (12 x 75 mm).
3. Pipettes delivering: 100 µL, 0.5 mL & 2.0 mL.
4. Distilled water.
5. Diagen PCA Ratio Kit (APCR390).

#### Procedure

##### Technique (Manual)

- 1) To duplicate **prewarmed** tubes or cuvettes at 37°C, add 100 µL of test (or control) sample.
- 2) To one tube add 100 µL of APTT reagent and to the duplicate tube 100 µL of PCA.APTT reagent.
- 3) Incubate both for 5 min. at 37°C.
- 4) Add 100 µL of 25 mM CaCl<sub>2</sub> / saline to both tubes and record the clotting time.
- 5) Calculate the PCA.APTT/APTT clotting time ratio (PCA ratio).

#### Please Note

When using the Diagen PCA ratio kits\* it is important to use the Calcium chloride provided, which contains saline. Omission of saline results in long normal clotting times for the PCA reagent.

#### Expected Values

With the Diagen APC resistance test systems\* using the manual technique, in our hands, gives a ratio of less than 2.2

\* Diagen PCA ratio screening test & Diagen PCA ratio in factor V depleted plasma kits.









#### Storage and stability

The unopened freeze dried vials are **best stored deep frozen**, but may be stored for up to 3 years at 2 - 8°C without deterioration. Once reconstituted the contents of the vial are then stable for up to 8 hours when held at 2 - 8°C.

#### Packaging

10 x 0.5 mL vials

#### Key guide to symbols

 REF	Manufacturers catalogue number.	 i	Consult instructions for use.
 LOT	Manufacturers batch number.	 Recon.	Requires reconstitution.
 IVD	For <i>in vitro</i> diagnostic use only.		Product expiry date.
	Biological risks.		Store at 4°C or below. Best stored deep frozen.



Manufacturer.

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