

Coatest® APC™ Resistance
Coatest® APC™ Resistance V



CHROMOGENIX

APC Resistance

Tracing Thrombosis... ...from phenotype to genotype

Resistance to activated protein C (APC) is the most common inherited risk factor for venous thrombosis [1-2]. Since APC resistance was first detected in 1993 [3], Chromogenix has been the leading company in APC resistance diagnostics.

Our Coatest APC Resistance test was a revolution in coagulation testing. Today, it is known that over 90% of cases of APC resistance are explained by a single point mutation in coagulation factor V (termed FV:Q⁵⁰⁶ or factor V Leiden [4-5]). Therefore, in addition to our original test we have also developed the factor V mutation screening test Coatest APC Resistance V.

With these tests, Chromogenix can offer the optimal combination of phenotypic and genotypic information for clinical decision making.

Coatest[®] APC[™] Resistance

For detection of the APC Resistance Phenotype

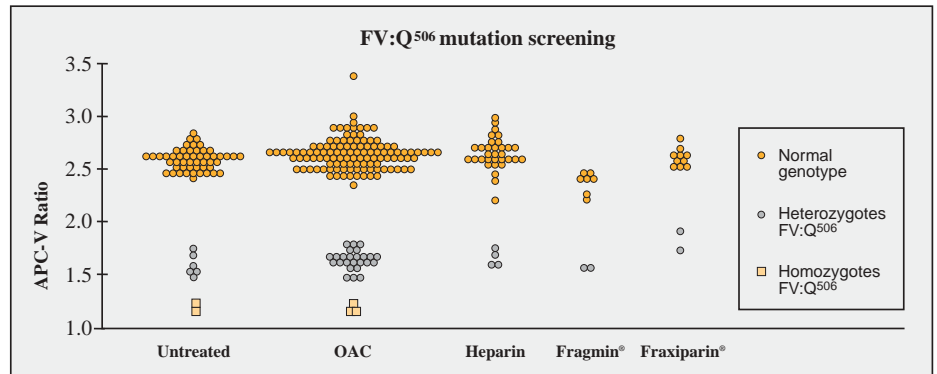
The original Coatest APC Resistance test detects the APC resistance phenotype regardless of its cause. The test principle is to perform two activated partial thromboplastin time (APTT)-reactions, one in the presence of exogenous APC and the other in its absence. The relationship between the two clotting times is expressed as an APC ratio.

In case of a normal APC response the clotting time is significantly prolonged in the presence of APC due to the down-regulation of thrombin formation. This is indicated by APC ratios in the range 2-5. However, in individuals with the APC resistance phenotype the APC ratios are below or equal to about 2 (dependent upon instrumentation [6]).

The APC ratio, as measured with Coatest APC Resistance, gives an overall estimation of the anticoagulant effect of APC and thus provides information on the thrombotic risk associated with both inherited and acquired APC resistance.

Test limitations: abnormal APTT, vitamin K antagonist therapy, heparin therapy.





The Coatest APC Resistance V method was used for analysis of plasma from various sample categories. A complete discrimination was obtained between normal and mutated factor V genotypes.¹¹ OAC= Oral Anticoagulant Therapy.

Coatest® APC™ Resistance V

For detection of APC Resistance due to Factor V Mutations

In order to allow for APC resistance analysis of plasma from patients on oral anticoagulant or heparin therapy, a modification of the original APC resistance test has been developed [7-10]. The modification is incorporated into the Coatest APC Resistance V kit and involves a 1+4 predilution of samples with Chromogenix unique V-DEF Plasma reagent [11]. This assay variant not only gives a 100% sensitivity* for the FV:Q⁵⁰⁶ mutation, but also strongly reduces the influence of pre-analytical variations such as plasma handling and storage [12,13]. In addition, Coatest APC Resistance V detects the FV:T³⁰⁶ mutation, a recently discovered although rare cause of APC Resistance [14].

Test results are expressed as a factor V related APC ratio (APC-V ratio), calculated as in the original test. APC-V ratios are generally lower than APC ratios obtained with the original APC resistance test. Typical median values are 2.6 (normal genotype), 1.7 (heterozygous FV:Q⁵⁰⁶) and 1.2 (homozygous FV:Q⁵⁰⁶).

Coatest APC Resistance V exclusively detects inherited APC resistance due to mutations in the factor V gene.

*Determined on Thrombolyzer (n=447), ACL (n=295), ST-4 (n=248) and MLA/Electra (n=50).

APC resistance

- Blood coagulation abnormality characterised by poor anticoagulant response to activated protein C.
- Major risk factor for thrombosis.
- Over 90% of cases explained by a point mutation in the factor V gene (Arg⁵⁰⁶ to Gln) which causes an impaired APC inactivation of factor Va.
- Autosomal dominant mode of inheritance.
- Trait is found approximately:
 - 5% of the general population,
 - 20% of unselected DVT patients,
 - 30% of patients with thrombosis occurring during oral contraceptive use,
 - 30% of patients developing DVT postoperatively,
 - 50% of patients with familial thrombophilia,
 - 60% of patients with thrombosis during pregnancy.



Coatest® APC™ Resistance

- Detects acquired and inherited APC resistance
- Low APC ratio indicates an increased thrombotic risk

Assay kits	CaCl ₂ [mL]	APTT [mL]	APC/CaCl ₂ [mL]	Control Plasma L1 + L2	Art. No.
Coatest® APC™ Resistance	1 x 8	1 x 16	4 x 2		82 26 43
Coatest® APC™ Resistance-C	1 x 8	1 x 16	4 x 2	1 + 1	82 29 08
Coatest® APC™ Resistance-S	2 x 2	2 x 4	2 x 2		82 29 16
Coatest® APC™ Resistance-SC	2 x 2	2 x 4	2 x 2	1 + 1	82 29 24

S=small, C=with control plasma

Coatest® APC™ Resistance V

- 100% sensitivity for the FV:Q⁵⁰⁶ mutation
- Applicable to oral anticoagulant and heparin treated patients

Assay kits	V-DEF [mL]	CaCl ₂ [mL]	APTT [mL]	APC/CaCl ₂ [mL]	Control Plasma L1 + L2	Art. No.
Coatest® APC™ Resistance V	4 x 4	1 x 8	1 x 16	4 x 2	1 + 1	82 31 20
Coatest® APC™ Resistance V-S	2 x 4	2 x 2	2 x 4	2 x 2	1 + 1	82 31 38
Reagent kit						
V-DEF Plasma	5 x 4					82 31 46

S=small

Controls

Kits	Control Plasma [mL]	Art. No.
Control Plasma Level 1 (Normal)	5 x 1	82 26 50
Control Plasma Level 2 (Abnormal)	5 x 1	82 26 68

References

1. Koster T, et al. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet* 342, 1503-1506 (1993).
2. Svensson P J. and Dahlbäck B. Resistance to activated protein C as a basis for venous thrombosis. *N Eng J Med* 330, 517-522 (1994).
3. Dahlbäck B, et al. Familial thrombophilia due to a previously unrecognized mechanism characterised by poor anticoagulant response to activated protein C: Prediction of a cofactor to activated protein C. *Proc Natl Acad Sci* 90, 1004-1008 (1993).
4. Bertina R M, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 369, 64-67 (1994).
5. Zöller B, et al. Identification of the same factor V gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C. *J Clin Invest* 94, 2521-2524 (1994).
6. Rosén S, et al. Multicenter evaluation of a kit for activated protein C resistance on various coagulation instruments using plasma's from healthy individuals. *Thromb Haemost* 72, 255-260 (1994).
7. Jorquera JI, et al. Modified test for activated protein C resistance. *Lancet* 344, 1162-1163 (1994).
8. Trossaert M, et al. Modified APC resistance assay for patients on oral anticoagulants. *Lancet* 344, 1709 (1994).
9. Dahlbäck B, et al. Modified APC-resistance test offers increased sensitivity and specificity for the FV:Q⁵⁰⁶ allele. *Thromb Haemost* 74, 1380-1381 (1995).
10. Trossaert M, et al. The modified APC resistance test in the presence of factor V deficient plasma can be used in patients without oral anticoagulant. *Thromb Haemost* 75, 521-522 (1996).
11. Hall C, et al. Evaluation of a modified APTT-based method for determination of APC resistance in plasma from patients on heparin or oral anticoagulant therapy. *Thromb Res* 89, 203-209 (1998).
12. Rosén S, and Sturk A. Activated Protein C Resistance - A major Risk Factor for Thrombosis. *Eur J Chem Clin Biochem* 35 (7), 501-516 (1997).
13. Freyburger G, et al. Response to activated protein C upon storage of whole blood and plasma. *Thromb Res* 93 (2), 89-95 (1999).
14. Williamson D, et al. Factor V Cambridge: A new mutation (Arg³⁵⁶->Thr) associated with resistance to activated protein C. *Blood* 91, 1140-1144 (1998).

Your Local Distributor is: DiaPharma Group, Inc.
8948 Beckett Road
West Chester, OH 45069

1-800-526-5224 (to order)
1-800-447-3846 (tech support)
(513) 860-9635 (fax)

<http://www.diapharma.com> (web)
info@diapharma.com (e-mail)

Chromogenix -
Instrumentation Laboratory SpA
Viale Monza, 338 - 20128 Milan, Italy
<http://www.chromogenix.com>

CHROMOGENIX

An Instrumentation Laboratory Company