REF **5344510** **APC Resistance Kit, 120T.** ENG

REF **5344515** **APC Resistance Kit, 40T.**

REF **5344512** **APC Control Kit, 2 x 1 mL**

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APC Resistance - English

INTENDED USE

APC Resistance Kit is a plasma based functional assay for the determination of resistance to activated protein C caused by the Factor V Leiden mutation (FV:Q^{506}).

SUMMARY

Activated protein C (APC) resistance is the most frequent hereditary defect associated with deep vein thrombosis. Over 95 % of the APC resistance phenotype can be explained by the Factor V Leiden mutation. This defect is caused by point mutation in the factor V gene resulting in a replacement of the amino acid Arg 506 by a Gln residue.The heterozygous (het) defect is associated with a 5 to 10 fold, the homozygous (hom) defect with a 50 to 100 fold increased thrombosis risk. There are two possibilities of detecting factor V (FV) Leiden. Plasma based functional assays identifying the phenotype expression of the defect or genotype determination, which can be done by PCR technology.

APC Resistance Kit is a plasma based functional clotting assay and differs from other functional APC resistance tests by acting specifically on the prothrombinase complex level. It is based on a FV-dependent prothrombin activator isolated from snake venom. Robustness and specificity of the assay is enhanced by elimination of possible disturbing influences by factors upstream the coagulation cascade and independency from calcium. Interference from UFH, LMWH and pentasaccharides in the blood sample is precluded by a heparin inhibitor added to reagents 1 and 2. Sample plasma is pre-diluted with reagent 4 (dilution plasma) and incubated at 37 °C with FV activator from snake venom (RVV-V from *Daboia russelli*). Coagulation is triggered by the addition of a FV dependent prothrombin activator from snake venom from *Notechis scutatus scutatus* in the absence of calcium. The clotting times are recorded and the ratios (clotting time in the presence of APC / clotting time in the absence of APC) are calculated.

REAGENTS

The APC Resistance Kit contains:

	Reagent / Content	Description
3 x 2 mL for 120 T. 1 x 2 mL for 40 T.	**R1** APC / RVV-V (+APC)	APC, RVV-V, Polybren, Hepes, BSA; lyophilized
3 x 2 mL for 120 T. 1 x 2 mL for 40 T.	**R2** RVV-V (-APC)	RVV-V, Polybren, Hepes, BSA; lyophilized
3 x 4 mL for 120 T. 1 x 4 mL for 40 T.	**R3** PTA Reagent	Prothrombin Activator, EDTA, Hepes, BSA; lyophilized
3 x 2 mL for 120 T. 1 x 2 mL for 40 T.	**R4** Dilution Plasma	Human Plasma, processed; lyophilized
1 x 1 mL	**C1** FV-L Negative Control*	Human Plasma; lyophilized
1 x 1 mL	**C2** FV-L Heterozygous Control*	Human Plasma; lyophilized

*Additionally, the controls are sold separately as APC Control Set 2 x 1 mL (REF 5344512).

Material required (not supplied with the kit)

- Distilled water
- Precision pipettes
- Laboratory timer

Warning and precautions

- RUO for research use only.
- This kit is intended for use by personnel trained in laboratory procedures and universal precautions for the use of chemicals and potentially biohazardous substances must be applied.
- All human blood or plasma products as well as test samples must be considered as potentially infectious. They have to be handled with appropriate care and in strict observance of safety regulations. The rules pertaining to disposal are the same as applied to disposing hospital waste.
- R1, R4 and control plasmas are made from human blood and any individual plasma involved in the procedure is tested HbsAg, HIV 1/2 Ab and HCV-Ab-negative. However, all human blood products should be handled as potentially infectious material.
- Get a Material Safety Data Sheet for this product from www.technoclone.com.

Symbol	Warning and Precausion	Product
	H302 Harmful if swallowed. H332 Harmful if inhaled. P260 Do not breathe dust. P314 Get medical advice/attention if you feel unwell.	R3 PTA Reagent
	H373 May cause damage to organs through prolonged or repeated exposure. Contains Disodium ethylenediaminetetraacetic acid	

Stability and storage

The expiry date printed on the labels is only applicable to storage of the unopened containers at 2…8 °C.

Stability opened/ in use:

Reagent	15...25 °C (on-board)	2…8 °C	< -20 °C
R1 APC / RVV-V (+APC)	24 hours	14 days	6 months
R2 RVV-V (-APC)	24 hours	14 days	6 months
R3 PTA Reagent	24 hours	14 days	6 months
R4 Dilution Plasma	24 hours	14 days	6 months
C1 FV-L Negative Control	8 hours	8 days	6 months
C2 FV-L Heterozygous Control	8 hours	8 days	6 months

Reagents and control plasmas material can be frozen once, in its original vial. Frozen material should be thawed at 37 °C and gently mixed before use.

TEST PROCEDURE

Preparation of plasma samples

Collect nine parts of freshly drawn venous blood in one part trisodium citrate (3.2 %). Refer to CLSI Document H21-A5 for instructions on specimen collection, handling, and storage.

Thaw frozen samples rapidly at 37 °C and centrifuge and separate if necessary. Gently mix before testing. After thawing, the assay must be performed within 2 hours. Samples may be frozen once.

Stability of undiluted samples (plasma):

-80 °C	-20 °C	2...8 °C	15...25 °C
at least 1 year	2 months	24 hours	4 hours

Preparation of reagents

Before starting the test, all the required components must be brought to room temperature.

Avoid foam formation when reconstituting plasmas and mixing reagents or buffers.

- ***R1** APC / RVV-V (+APC):* Reconstitute each vial with 2.0 mL of distilled water. Allow the reconstituted material to stand at room temperature for 30 minutes before use. Swirl to mix before use.
- ***R2** RVV-V (-APC):* Reconstitute each vial with 2.0 mL of distilled water. Allow the reconstituted material to stand at room temperature for 30 minutes before use. Swirl to mix before use.
- ***R3** PTA Reagent:* Reconstitute each vial with 4.0 mL of distilled water. Allow the reconstituted material to stand at room temperature for 30 minutes before use. Swirl to mix before use.
- ***R4** Dilution Plasma:* Reconstitute each vial with 2.0 mL of distilled water. Allow the reconstituted material to stand at room temperature for 30 minutes before use. Swirl to mix before use.
  Attention: Extended incubation of reagent R4 may – due to its high protein content – cause a phase separation characterized by a clear solution with a fine, whitish layer on its surface. This may be erroneously interpreted as coagulation. Therefore, the reagent must absolutely be brought in its initial homogeneous and slightly cloudy form just before use.
- ***C1** FV-L Negative Control:* Reconstitute each vial with 1.0 mL of distilled water. Allow the reconstituted material to stand at room temperature for 30 minutes before use. Swirl to mix before use.
- ***C2** FV- L Heterozygous Control:* Reconstitute each vial with 1.0 mL of distilled water. Allow the reconstituted material to stand at room temperature for 30 minutes before use. Swirl to mix before use.

Performance of the test

Use on Ceveron alpha / Ceveron 100 series instruments

The respective application for APC-R is pre installed on Ceveron alpha and 100 series instruments.

Use on Automated coagulation instruments

Technoclone provides instrument specific application sheets which contain analyser / assay specific handling and performance information which may differ from that provided in this instruction for use. In this case the information contained in the application sheets supersedes the information in this instruction for use.

Use on semi-automated coagulation instruments

Prepare reagents as described above. Thaw frozen samples as described above ensuring negligible loss of activity of labile coagulation factors and absence of cryoprecipitate. Invert thawed sample for homogenization. Determine +APC clotting time (clotting time in the presence of Activated Protein C), -APC clotting time (clotting time in the absence of Activated Protein C) and calculate the ratio according to the following scheme:

		+ APC	**- APC**
	Sample or control Plasma	30 µL	30 µL
R4	**Dilution Plasma**	20 µL	20 µL
		mix prior to use	mix prior to use
R1	**APC/RVV-V (+ APC) Reagent**	50 µL	-
R2	**RVV-V (-APC) Reagent**	-	50 µL
	Incubation	3 min, 37 °C	3 min, 37 °C
R3	**PTA Reagent**	50 µL	50 µL
		Determine clotting time	Determine clotting time
	Ratio calculation	+ APC clotting time Ratio = ---------- - APC clotting time	

LIMITATION OF THE TEST

No significant differences are observed when fresh or frozen plasma samples are used. It does neither matter whether buffered or un-buffered citrate plasma is used.

There is no significant influence on ratio or test sensitivity in case of Fibrinogen, Prothrombin, FVIII, FX, ATIII, Protein C, or Protein S deficiency (up to 100 %) or excess of Fibrinogen, FVIII, ATIII, or TFPI (up to 5 times normal value). Lupus anticoagulant antibodies did not influence the test. But a high Factor V deficiency (<50 %) may lead to strongly elevated clotting times and thus may lead to loss of discrimination performance. The presence of Aprotinin (which inhibits the APC used in this test) and Protamine in the specimen's blood can considerably shorten the clotting times, which may also lead to loss of discrimination power.

Due to the addition of Polybrene the prescribed assay procedure allows for the analysis of plasma from anticoagulated plasma at heparin levels ≤2 IU/mL (UFH and LMWH) or pentasaccharide levels ≤2 µg/mL. The effect of direct thrombin inhibitors such as Hirudin or Argatroban is not inhibited by Polybrene. Hirudin in the plasma has a strong effect on clotting times and thus precludes proper discrimination of the different genotypes.

Therefore, after treatment of with Aprotinin, Protamine or direct thrombin inhibitors, it is recommended to either wait at least 24 hours before blood sampling for the test, or determine the FVL by a PCR method.

INTERPRETATION OF RESULTS

Differentiation of homozygous, heterozygous and negative samples is based on the typical ratio ranges measured with genotyped plasma samples.

Typical ratio ranges for PCR-genotyped plasma samples on different devices are shown in the table below.

KC-4/-10 A Micro		
Genotyp FV:Q^{506}	n	Ratio Range (min/max)
Negative	99	> 3.0
Heterozygous	166	1.3 – 1.9
Homozygous	25	0.9 – 1.1

BCS / BCS XP		
Genotyp FV:Q^{506}	n	Ratio Range (min/max)
Negative	143	> 3.0
Heterozygous	170	1.4 – 2.2
Homozygous	27	0.9 – 1.1

CS line		
Genotyp FV:Q^{506}	N	Ratio Range (min/max)
Negative	62	> 3.5
Heterozygous	37	1.4 – 2.0
Homozygous	2	1.0 – 1.1

ACL Top Family		
Genotyp FV:Q^{506}	n	Ratio Range (min/max)
Negative	138	> 2.8
Heterozygous	94	1.3 – 1.8
Homozygous	1	1.0 – 1.1

STA line		
Genotyp FV:Q^{506}	n	Ratio Range (min/max)
Negative	134	> 2.9
Heterozygous	83	1.3 – 1.8
Homozygous	27	0.9 – 1.1

Ceveron alpha / Ceveron 100 series		
Genotyp FV:Q^{506}	n	Ratio Range (min/max)
Negative	58	> 2.7
Heterozygous	31	1.35 – 2.1
Homozygous	2	0.9 – 1.25

It is recommended to determine laboratory specific ratio ranges with genotyped plasma samples.

Attention: Factors mentioned under "Limitations and Interferences" can change the specific ratio ranges, so that differentiation of samples become impossible.

QUALITY CONTROLS

Negative control or wild-type (neg) shows normal response to APC whereas heterozygous control (het) shows response to the presence of the heterozygous type of Factor V Leiden mutation. A control run should be made with each test series. If values outside the certified range (ratio) are obtained, a complete check of reagents should be made and the analysis should be repeated. If the problem persists, a complete instrument check should be made and the analysis should be repeated.

Device:	C1 FV-L-Negative Control*	C2 FV-L Heterozygous Control*
KC-4/-10 A Micro	3.8 – 11.9	1.3 – 1.8
BCS XP	3.6 – 6.9	1.3 – 2.1
CS-line	3.8 – 7.9	1.5 – 1.8
ACL TOP -Family	3.5 – 7.5	1.3 – 2.0
STA -line	2.6 – 9.3	1.4 – 1.7
Ceveron alpha / Ceveron 100 series	2.7 – 5.1	1.3 – 2.1

These ranges are valid for all lots of control plasmas.

If values outside these ranges are obtained the test results are not valid and shall not be used. When controls are used in combination with another suitable test or instrument expected ratio values may be different and have to be determined locally under appropriate conditions.

PERFORMANCE CHARACTERISTICS

Performance data are given below. Results obtained in individual laboratories may differ.

Specificity and Sensitivity

With the samples tested so far APC Resistance Kit provided 100 % sensitivity and 100 % specificity for carriers of heterozygous and homozygous FV:Q^{506} mutation as determined by BCS XP (n=340), KC-4/-10 A micro (n=290), CS-2100/CS-5100 (n=101), ACL Top 500 (n=233) and STA C (n=244). Due to the functional detection technique the assay is supposed to detect other FV mutations leading to APC-R phenotype as well. However their prevalence is very low compared to the FV Leiden mutation.

Accuracy and Reproducibility

With 2 genotyped plasma samples (neg/het) a series of 25 measurements were taken on the same day on 2 different fully automated analytical systems (BCS, CA-500). Correlation of variance (CV) was determined based on the ratio. For both instruments and plasma genotypes the CV was below 5 %.
In a further study on the STA Compact analyzer, a series of 10 tests on each of 5 consecutive days were done using a heterozygous and a negative (wild-type) control. The CV of the clotting times and the ratios within each day and over all 5 days was ≤5.0 for the negative control and <3.0 for the heterozygous control.
The values obtained during these 5 days were within the following ranges:

	C1 FV-L Negative Control			C2 FV-L Heterozygous Control		
	+APC(s)	-APC(s)	Ratio	+APC(s)	-APC(s)	Ratio
min	132.9	24.2	5.3	39.2	25.4	1.5
max	166.3	25.7	6.7	42.6	27.7	1.6

LITERATURE

Please contact Technoclone www.technoclone.com or your local distributor.

EDITORIAL NOTE

This document is available in several languages. The translations have been done using the master document in English. In the event of doubts or discrepancies, the wording in the master document in English shall take precedence.

 Manufacturer

RUO Research use only

 Storage temperature

LOT Lot

 Expiry

REF Catalogue

 Consult instructions for use

GTIN Global Trade Item Number

 Biological risk

AQUA Distilled

 Manufacturing date

CONT Control

DIL Dilute or disolve in

Σ Determinations

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