

# Thrombotest™ and Thrombotest™ Automated



## AVAILABLE PRODUCTS

Thrombotest™ 6x11 mL	240 tests
Thrombotest™ 12x2.2 mL	96 tests
Thrombotest™ Automated 6x11 mL	240 tests
Calcium Chloride 3.2 mmol/L	6x12 mL

## DISTRIBUTORS



**t NL** +31 (0)71 - 523 10 50  
**t B** +32 (0)2 - 426 85 12    **e** kordia@kordia.com  
**t F** +33 (0)9 - 65 37 05 75    **i** www.kordia.com

## SYMBOLS/ SYMBOLE/ SYMBOLES/ SIMBOLI/ SÍMBOLOS/ SYMBOLEN/ ΣΥΜΒΟΛΑ

**CE** Conformity to the IVD directive 98/79/EC  
 Geprüft nach IVD Direktive 98/79/CE  
 Conforme à la directive IVD 98/79/CE  
 Conforme alla direttiva IVD 98/79/CE  
 De conformidad con la directiva IVD 98/79/EC  
 Conform IVD richtlijn 98/97/EC  
 Συμμόρφωση με την οδηγία 98/79/ΕΚ για IVD προϊόντα

**IVD** In Vitro Diagnostic Medical Device  
 In Vitro Diagnostikum  
 Dispositif médical de diagnostic in vitro  
 Dispositivo medico-diagnostico in vitro  
 Dispositivo médico de diagnóstico in vitro  
 Medisch hulpmiddel voor in-vitro diagnostiek  
 In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν

**i** Instructions for use.  
 Gebrauchsanweisung  
 Mode d'emploi  
 Guía de uso  
 Istruzioni per l'uso  
 Gebruiksaanwijzing  
 Οδηγίες χρήσεως

**≤ +20°C** Storage temperature  
 Lagerungstemperatur  
 Température de conservation  
 Temperatura di conservazione  
 Temperatura de almacenaje  
 Opslag temperatuur  
 Θερμοκρασία αποθήκευσης

**🕒** Expiration date (year-month)  
 Verfallsdatum (Jahr-Monat)  
 Date de péremption (année-mois)  
 Data di scadenza (anno-mese)  
 Fecha de vencimiento (año-mes)  
 Niet gebruiken na (jaar-maand)  
 Ημερομηνία λήξης (έτος-μήνας)

**LOT** Lot number  
 Lotnummer  
 Numéro de lot  
 Numero di Lotto  
 Lote número  
 Lot nummer  
 Αριθμός παρτίδας

**REF** Catalogue number  
 Katalognummer  
 Numéro de code du produit  
 Codice prodotto  
 Catálogo número  
 Catalogus nummer  
 Αριθμός καταλόγου

**🏭** Manufacturer  
 Hersteller  
 Fabricant  
 Produttore  
 Fabricante  
 Fabrikant  
 Κατασκευαστής

A distributor list is available at [www.axis-shield-poc.com](http://www.axis-shield-poc.com)  
 Eine Distributorenliste kann unter [www.axis-shield-poc.com](http://www.axis-shield-poc.com) gefunden werden  
 Une liste des distributeurs est disponible sur le site [www.axis-shield-poc.com](http://www.axis-shield-poc.com)  
 Per un elenco dei distributori, consultare il sito [www.axis-shield-poc.com](http://www.axis-shield-poc.com)  
 Una lista de distribuidores está disponible en [www.axis-shield-poc.com](http://www.axis-shield-poc.com)  
 Een lijst van distributeurs kan worden gevonden op [www.axis-shield-poc.com](http://www.axis-shield-poc.com)  
 Μπορείτε να βρείτε τον κατάλογο διανομέων στη διεύθυνση [www.axis-shield-poc.com](http://www.axis-shield-poc.com)



**AXIS-SHIELD PoC AS**

P.O. Box 6863 Rodeløkka  
 NO-0504 Oslo, Norway  
[www.axis-shield-poc.com](http://www.axis-shield-poc.com)

ISO 9001 and ISO 13485 certified company

# Thrombotest™ and Thrombotest™ Automated



## PRODUCT DESCRIPTION

### INTENDED USE

Thrombotest™ is an *in vitro* combined PT reagent to be used for control of oral anticoagulant therapy. Thrombotest™ Automated is the same reagent as the ordinary Thrombotest reagent, but Thrombotest™ Automated is especially designed to be used with coagulation instruments based on optical end point reading.

### TEST PRINCIPLE

Thrombotest™ and Thrombotest™ Automated (hereafter called Thrombotest™) are combined Prothrombin Time (PT) tests. Thrombotest™ is lyophilized and contains bovine brain thromboplastin and adsorbed bovine plasma. The thromboplastin is free from coagulation factors and intermediates. The adsorbed plasma is added as a source of factor V and fibrinogen.

Thrombotest™ is sensitive to the coagulation factors II, VII and X. The test is also sensitive to the PIVKA Proteins (Protein Induced by Vitamin K Absence or Antagonists). When Thrombotest™ reagent is mixed with plasma or blood, factor VII will be activated and ultimately, a clot will be formed.

### PRESENTATION

Thrombotest™: 12x2.2 mL (96 tests)  
6x11 mL (240 tests)  
Thrombotest™ Automated: 6x11 mL (240 tests)

### MATERIAL REQUIRED (not supplied with the kit)

- Pipettes: 30 µL, 50 µL, 100 µL, 250 µL, 2.2 mL and 11 mL
- Solutions: Calcium Chloride 3.2 mmol/L, Distilled water

### WARNINGS AND PRECAUTIONS

- **[IVD]** For *in vitro* diagnostic use.
- Thrombotest™ contains no human material
- Thrombotest™ has been derived from norwegian healthy bovine animals approved for human consumption. Norway is known to be free from the most contagious diseases, and Bovine Spongiform Encephalopathy (BSE) has never been reported in Norway.

### ANALYTICAL SPECIFICITY

Thrombotest™ contains bovine thromboplastin as activator of the coagulation process and factor V and fibrinogen are added. Thrombotest™ is sensitive to the concentration of factors II, VII and X in the sample to be measured.

### STANDARDISATION

The coagulation activity is expressed as percentage of normal value or in INR units (International Normalized Ratio). The normal value represents the mean of an adult population.

Each lot is calibrated against the International Reference Preparation for bovine thromboplastin OBT/79 through a house reference lot of Thrombotest™ according to WHO recommendations. OBT/79 is in turn standardized against the primary International Reference Preparation (NIBSC code 67/40).<sup>1</sup>

Each lot is supplied with a correlation table for manual testing with INR and percent (%) activity, and with the specific International Sensitivity Index (ISI) for manual testing.

### MEASURING RANGE

It is recommended to use Thrombotest™ in the range from 100 % to 3% coagulation activity or from INR= 1.0 to INR = 8.0.

### PRECISION

A coefficient of variation (CV) of 1- 2 % (within runs) is observed for Thrombotest™ tested with Control Plasma AK on the Thrombotrack™ 1 (250-rpm).

### LIMITATION OF THE TEST

Only citrate should be used as anticoagulant (neither EDTA nor heparin).

Corrections for hematocrit deviation: See "Interpretation of Results".

Activation of the coagulation system will induce considerable shortening of the clotting time. Sample tubes used should be made from "non-activating" material and contain correct citrate concentration. Testing should be performed at 37 °C.

Cold activation of the plasma samples should be avoided.

## STABILITY AND STORAGE

### Thrombotest™:

Unopened vials: ≤ 20 °C: 2 years  
37 °C: 1 hour  
Reconstituted reagent: 15-25 °C: 10 hours  
2-8 °C: 3 days  
≤ 20 °C: 2 months

Deep-frozen reagent should be thawed for at least 10 minutes at 37 °C in a waterbath or in a heating block at 37 °C for 15 minutes.

### Thrombotest™ Automated:

Unopened vials: ≤ 20 °C: 2 years  
37 °C: 1 hour  
Reconstituted reagent: 15-25 °C: 10 hours  
2-8 °C: 3 days

**NB! Reconstituted Thrombotest™ Automated can not be frozen.**

The expiry date printed on the labels applies to storage of the unopened bottles at ≤ 20 °C.

Upon storage, caps should be screwed tightly.

## TEST PROCEDURE

### BLOOD SAMPLE COLLECTION

#### Venous blood

Venous blood is collected in evacuated siliconized blood collection tubes for coagulation analysis (0.11 or 0.13 mol/L Na<sub>2</sub>-citrate).

Alternatively, 1 volume of 0.11 or 0.13 mol/L Na<sub>2</sub>-citrate is added to 9 volumes of whole blood in a non-activating tube.

#### Preparation of plasma

After centrifugation, measure 30µL of the supernatant plasma into a pipette. Alternatively, dilute 6 volumes of plasma with 4 volumes of saline in a plastic or siliconized glass tube. Measure 50 µL of the diluted plasma into a pipette.

#### Capillary blood

Make a skin puncture sufficiently deep to produce a free flow of blood. The first drop of blood should always be used.

#### Preparation of citrated capillary blood<sup>4</sup>

Use a 0.1 mL pipette with graduation at 50 µL.

1. Draw 50 µL of diluted Na<sub>2</sub>-citrate solution (1 volume of 0.11 mol/L or 0.13 mol/L Na<sub>2</sub>-citrate + 8 volumes of physiological saline) into the pipette.
2. Fill the pipette to a volume of 0.1 mL with capillary blood. The first drop of blood should be used.
3. Transfer the mixture of blood (50 µL) and diluted Na<sub>2</sub>-citrate (50 µL) to the test tube.

### RECONSTITUTION OF THE REAGENT

Method	Liquid	Large vials	Small vials
Venous blood	3.2 mmol/L CaCl <sub>2</sub>	11 mL	2.2 mL
Plasma	»	»	»
Citrated capillary blood	»	»	»
Capillary blood	Distilled water	»	»

### IMPORTANT!

- The reconstitution liquid and reagent have to reach room temperature before mixing.
- The reconstitution liquid is added to the lyophilized reagent.
- Shake *vigorously* immediately.
- The reagent is ready for use after 5 minutes.

### TEST PROCEDURE, MANUAL METHOD

Method	Reconstituted Reagent	Sample
Venous blood	250 µL	50 µL
Plasma	250 µL	30 µL
Diluted plasma	250 µL	50 µL
*Citrated capillary blood	250 µL	100 µL
Capillary blood	250 µL	50 µL

- Pipette 250 µL of reagent into small clotting tubes and prewarm in a waterbath at 37°C for at least 3 minutes.
- Dispense the blood sample (for correct volume, see Table) from the pipette just above the surface of the reagent and simultaneously start the stopwatch.
- \* The citrated capillary blood sample should be incubated at 37 °C for 2-3 minutes. Pipette 250 µL of pre-warmed Thrombotest reagent into the sample, simultaneously starting the stopwatch.
- Mix blood and reagent by flicking the tube once or twice and leave the tube in the waterbath for about 30 seconds for normal blood and 50 seconds for blood from patients on oral anticoagulant therapy.
- At short intervals, tilt gently to observe and record the endpoint of coagulation.
- For the venous blood- and plasma methods, the coagulation activity is read from the column for venous blood or plasma in the Correlation table.
- For the capillary- and citrated capillary blood method, the coagulation activity is read from the column for capillary blood in the Correlation table.

### APPLICATION ON INSTRUMENTS

When using Thrombotest™ with Thrombotrack™ 1 (250 rpm), Thrombotrack™ Solo and Thrombotrack™ Select 2, the table for manual testing can be used for translation into % or INR values. When using Thrombotest™ on automatic instruments, an instrument specific ISI value must be established if the results should be reported in INR values. Axis-Shield offers a Calibration Set containing three different plasmas with INR values for Thrombotest™, to be used for establishing the ISI value and normal value on instruments. This calibration should be performed for every new lot of Thrombotest™.

### STORAGE AND STABILITY OF THE SAMPLE MATERIAL

- **Venous blood/plasma:**  
The samples should be stored in plastic tubes or non-activating vials. Blood samples from patients on oral anticoagulant treatment should be tested within 48 hours. Samples from normal individuals within 3 hours.
- **Capillary blood:**  
Capillary blood samples should be tested immediately.
- **Citrated capillary blood:**  
Samples should be transferred to plastic tubes. Samples from patients on oral anticoagulant treatment should be tested within 5 hours. Samples from normal individuals should be tested within 1 hour.

### INTERNAL QUALITY CONTROL

Axis-Shield offers Control Plasmas (Normal, Abnormal and AK) to control the performance of the test system used.

## INTERPRETATION OF RESULTS

### Normal range<sup>2</sup>

INR < 1.08

### Therapeutic range

For oral anticoagulant treatment indications and duration of treatment, please make reference to local guidelines.

### Correction for abnormal hematocrit

When using whole blood, the result depends on the hematocrit value. This can be corrected by multiplying the **percent value** by a factor. In practice, this is only necessary in cases of patients with severe anemia or polycythemia. Because of the PIVKA effect, there is a different factor for patients receiving oral anticoagulant therapy (AC-patients).

Hematocrit vol. %	Factor (normal)	Factor (AC-patients)
20	0.70	0.85
30	0.80	0.90
40	0.90	0.95
50	1.10	1.05
60	1.38	1.20
70	1.80	1.40

### Combined coumarin and heparin therapy

Administration of heparin as continuous intravenous drip in therapeutic doses has minimal influence on the Thrombotest™ time. However, combined therapy with coumarin and continuous intravenous heparin can be controlled with Thrombotest™ in the usual way. When the Thrombotest™ value has decreased to about 5% or INR 4.8, the heparin can be discontinued. The Thrombotest™ value will then increase by 2-5%, but still be within the therapeutic range. The optimal level of anticoagulation can be obtained by minor adjustments of the coumarin dosage.

### Influence of heparin on the Thrombotest™ value

By intermittent injection of heparin or LMWHeparin, the Thrombotest™ time is prolonged to varying degrees depending on the brand of heparin, the dosage and the individual sensitivity. After intravenous injection of 15,000 I.E. unfractionated heparin, the effect may last up to about 8 hours.

The prophylactic low dosage regime (5,000 I.E. 8 hourly) has no significant effect on the Thrombotest™ value.

In order to obtain correct Thrombotest™ values, the blood sample should not be collected until the heparin effect has vanished, or if collected earlier, adding 0.08 mg of protamine sulphate per mL blood can neutralize the heparin effect.<sup>3</sup>

For the control of ordinary heparin therapy, a standardized Activated Partial Thromboplastin Time (APTT) method (Cephotest™) is recommended.

## TROUBLESHOOTING

1. Always use the first drops of capillary blood. Do not use cotton wool before sampling because it initiates coagulation.
2. Use only clean pipettes, collecting and clotting tubes.
3. Use reconstitution liquid (Calcium Chloride or distilled water) at room temperature. Low temperature might cause flocculation of cold insoluble fibrinogen in the bovine plasma component of the reagent.
4. Control that the number on the Correlation table corresponds to the batch number of the vial.
5. Check that correct instrument specific ISI value is established by checking with Control Plasmas.

## BIBLIOGRAPHY

1. WHO Expert Committee on biological standardization. 48th. Report. WHO Technical Report 1999; Series 889.
2. Owen P.A.: Thrombotest. A new method for controlling anticoagulant therapy. Lancet II, 1959; 754-758.
3. Norday A.: Difference in the heparin - neutralizing effect of protamine and polybrene as tested by Thrombotest. Scand. J. clin. Lab. Invest. 1963; 15: 205-210.
4. Hjort P. F. et al.: Thrombotest with citrated capillary blood. Farmokoterapi, XVII, No. 1, 1961.