

Intended use: For In Vitro Diagnostic Use Only
 ap-TEM® is a ready-to-use ROTEM® system reagent for detection of hyperfibrinolysis and for a heparin-insensitive analysis of coagulation from citrated blood samples. ap-TEM® is always used in combination with ex-TEM®.

Reagents:
Product Name: ap-TEM®
Reference Number: REF 503-04
Package size: 10 x 1 vials ap-TEM® for 10 x 5 tests
Constituents:

Aprotinin, 0.2 mol/l CaCl₂ in HEPES buffer pH 7.4 and 0.1% sodium azide (NaN₃) in glass vials.
Preparation of the ready-to-use reagent:
 The reagent is ready to use after thorough but careful mixing.

Storage and Stability:
 Store at +2 to +8°C. The unopened reagent is stable until the expiry date indicated on the label.
Stability after Initial Use:
 Opened vials must be used within 14 days from first opening. Always record the date until which the opened reagent is stable in the designated field on the test tube. Store at +2 to +8 °C. Avoid contamination and always close the vials again after each use to avoid evaporation.

Additional Material:
 ROTEM® device; blood collection tubes (~0.106 M or ~0.129 M sodium citrate) for coagulation testing; cup and pin (measurement cells; REF 700005 / REF 400050); pipette tips (REF 400041 / REF 400040 / REF 400044), ex-TEM® reagent (REF 503-03) as activator.

Specimen:
 Freshly prepared citrated blood. Carefully mix 9 vol. of venous blood with 1 vol. of sodium citrate (~0.106 M or ~0.129 M sodium citrate) (1,2).

Method:
Analytical Principle:
 Fibrinolytic processes are detected by a loss of the clot firmness during the clot formation analysis with the ROTEM® (3). In the APTEM test activation is caused by ex-TEM® using the extrinsic system. The plasmin-antagonist Aprotinin prevents a fibrinolysis in vitro; CaCl₂ in the ap-TEM® recalcifies the sample. The APTEM test provides information regarding coagulation without fibrinolysis effects, EXTEM includes such effects. Evidence of fibrinolysis activity is obtained by comparing the results from the EXTEM and APTEM test.

In thromboelastometric measurements with ROTEM®, the clotting process is started after addition of the reagents to the sample and then continuously monitored by the ROTEM® analyser. Automatic analysis, calculation and documentation of the coagulation time (CT), clot formation time (CFT), maximum clot firmness (MCF), maximum lysis (ML) and other parameters, provide a record of the complete haemostasis regarding coagulation activation, clot formation, clot polymerisation and clot stability through to fibrinolysis (4).

A significant normalisation of the parameters CT, CFT and MCF in the APTEM test by comparison with the EXTEM test indicates hyperfibrinolysis. Parameter values differing from the established reference ranges indicate a possible coagulation disorder.

Measurement Calculation:
 The ROTEM® device offers various parameters. These parameters and their mathematical background are explained in the ROTEM® user manual.

Limitations of the Procedure:
 Always use freshly prepared citrated blood specimens if hyperfibrinolysis is suspected as inadequate stability of sample can be expected. Store citrated blood at room temperature, Do NOT store at +2 to +8°C (5). Before analysis, bring citrated blood samples to 37 °C and, immediately prior to use, mix carefully and thoroughly to eliminate storage sedimentation. Avoid foaming!
 Aspirin, Clopidogrel and von Willebrand Factor only have a very slight effect on this method. Abnormal patterns in the APTEM test may also be caused by the effect of anticoagulants such as hirudin. The effect of oral anti-coagulants (cumarin) has a secondary influence on the results compared with thromboplastin time. Abnormal APTEM results may be caused by using EXTEG L reagent as an activator. Heparin-insensitivity is partially achieved by the use of EXTEM as an activator. APTEM is not designed to be used with native blood.

Quality Control:
 A stable control with ongoing fibrinolysis activation is technically impossible. As ap-TEM® is always used together with ex-TEM® use of control materials is recommended for regular quality control of the EXTEM test (e.g. REF 503-21 RO-TROL N / REF 503-24 ROTROL N / REF 503-25 ROTROL P). Further information may be found in the respective instructions for using these materials.

Expected Values:
 The following reference values were obtained with samples from healthy Central European blood donors for ex-TEM® in ROTEM® (n=167): CT 38-79 sec, CFT 34-159 sec, α-angle 63-83 and MCF 50-72 mm. The values for ap-TEM® are within the same range. These values should only be used for guidance and must be viewed with caution! They are tentative and may vary from laboratory to laboratory, as they are dependent on the collection of blood samples and other pre-analytical factors. Confirmation of the values by a laboratory-/hospital-specific reference group is recommended.

Pathological Results:
 The diagnosis of fibrinolysis is resultant from comparison using ex-TEM® alone and with ex-TEM® and ap-TEM®. If in the EXTEM test pathological values, especially clot firmness (A-values/MCF) and lysis parameters in the APTEM test are corrected in part or in full, hyperfibrinolysis is probably present. If the parameters are not completely corrected, additional haemostasis disorders such as dilution coagulopathy and others should be considered. (see ex-TEM® package insert).

Research applications:
 Information regarding the use of the ap-TEM® reagents in conjunction with hep-TEM® for research purposes can be requested from Pentapharm GmbH.

Warnings: For in-vitro diagnostic use only.
Precautions:
 Human blood must be handled with care, following the precautions recommended for potentially infectious substances (6).

Procedure (APTEM assay):

- A. Mix the reagents carefully before use. Carefully reconstitute any sediment formed into suspension, especially in the case of ex-TEM®. Bring all reagents to room temperature before use (approx. 15 minutes for reagents taken from the refrigerator)
- B. Prepare a citrated blood sample, as recommended. Pre-heat the citrated blood to the measuring temperature.
- C. NOTE: Follow the ROTEM® user manual for correct operation of the device.
- D. Select a channel for the measurement.
- E. Remove cup & pin (measurement cell) together from the pack and place the pin (stamp) located in the cup firmly on to the measurement axis (avoid touching it).
- F. Insert the cup into the pre-warmed cup holder and press it firmly into place with the MC rod (REF 100017).

- **Automatic Pipetting:**
 Follow each on-screen instruction when performing the test using the automatic pipette.
- **Manual Pipetting:**
 Perform pipetting into the pre-warmed cup holder in the following sequence:
1. 20 µL ex-TEM® reagent.
 2. 20 µL ap-TEM® reagent (with new pipette tip).
 3. 300 µL citrated blood (pre-warmed; with new pipette tip).
 4. Begin the measurement with the appropriate command (e.g. Manual) in the desired pre-selected channel.
 5. Mix the sample and reagent by aspirating a 300 µL volume into the pipette once and slowly dispensing it.
 6. Finally, place the cup holder containing the sample mixture carefully and immediately on the appropriate channel.
 7. Stop the measurement at the desired time, remove the sample and dispose of it in conformity with local regulations.
 8. The channels may then be released for the next measurement using the appropriate.

Performance Data:

Precision:

	CT CV (%)	CFT CV (%)	α-angle CV (%)	A10 CV (%)
In series*	9	18	3	8

* 15 measurements on one single channel of the device
 Blood samples from healthy donors were used for the analysis.

Heparin responsiveness:

From approx. 0.3 U/mL heparin (whole blood mixed with UFH) in EXTEG L activated tests there is an extension of the initial CT value. At > 1 U/mL UFH (whole blood mixed with UFH), and in strongly heparinised patients (e.g. vascular/cardiosurgery) CT mostly reached values around 500-900 sec or the sample becomes uncoagulable (CT>15 min). When using ex-TEM® as an activator, the CT is unaffected up to a heparin concentration of 4 U/ml UFH in whole blood (corresponding to 8 U/mL in the plasma).

Bibliography:

- (1) NCCLS Document H3-A4 Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture Fourth Edition, Approved Standard (1998)
- (2) DIN 58905-1; Blutentnahme; Teil 1: Gewinnung von venösem Citratplasma für hämostaseologische Analysen.
- (3) Blutgerinnungsstudien mit der Thromboelastographie, einem neuen Untersuchungsverfahren. Hartert, H.; Klin. Wochenschrift 1948; 26: 577-583
- (4) Thromboelastographic Coagulation Monitoring during Cardiovascular Surgery with the ROTEG Coagulation Analyzer, Calatzis, A. et al., Management of Bleeding in Cardiovascular Surgery edited by Roque Pifarre; Hanley & Belfus, Inc. Philadelphia, PA, 2000
- (5) NCCLS Document H21-A2. Collection, transport, and processing of blood specimens for coagulation testing and performance of coagulation assays, 3rd ed. Approved Guideline 1998
- (6) Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services, Washington 1993 (HHS publication No. (CD) 93-8395)

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