# PENTAPHARM GmbH

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# fib-TEM®

# Instructions for Use

## Intended Use:

For In Vitro Diagnostic Use Only

fib-TEM® is a ready-to-use ROTEM® system reagent which allows an isolated assessment of the fibrinogen level and the quality of the fibrin polymerisation in citrated blood by inhibiting the thrombocytes. Contrasting with ex-TEM® it also allows assessment indirectly of the contribution of thrombocytes to coagulation. fib-TEM® is always used in conjunction with ex-TEM®.

## *Reagents:*

**Product Name:** fib-TEM®
**Reference Number:** REF 503-06
**Package size:** 10 x 1 vial fib-TEM® for 10 x 5 tests
**Constituents:**
Cytochalasin D / DMSO solution 0.2 mol/l $CaCl_2$ in HEPES buffer pH 7.4, preservative in glass vials.
**Preparation of the ready-to-use reagent:**
The reagent is ready to use after mixing gently but carefully.
**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 reagent vial. 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 & 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:**
The fib-TEM® reagent contains cytochalasin D as thrombocyte inhibitor (inhibiting the actin/myosin-system) and $CaCl_2$ as recalcification reagent. Therefore only the fibrin level of the coagulation is measured on ROTEM® in an extrinsic activated test using ex-TEM®, the platelet level is inactivated (3). Therefore, using the FIBTEM test the quality of fibrin polymerisation or the fibrinogen concentration can be estimated quickly. A weak fibrin coagulation with fib-TEM® indicates fibrinogen deficiency or a disturbance in fibrin polymerisation.
By means of a parallel test using only ex-TEM® the contribution of thrombocytes to coagulation is also recorded. The difference in clot firmness between FIBTEM and the EXTEM test is an indirect measure of the thrombocyte function.
In thromboelastometric measurements with ROTEM®, the clotting process is started after addition of reagents to the sample and then continuously monitored by the ROTEM® analyser. Calculation and documentation of coagulation time (CT), clot formation time (CFT), maximum clot firmness (MCF) and other parameters is automatic, providing a record of all aspects of the haemostasis regarding coagulation activation, clot formation, clot polymerisation and clot stability through to fibrinolysis (4, 5).
For the assessment of clot formation in the FIBTEM test, reference is made chiefly to firmness parameters such as A10, A20, MCF. CT and CFT are of secondary importance.
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.
**Limitation of the Procedure:**
Always use freshly prepared citrated blood specimens. In ex-TEM® -based tests of blood samples from healthy subjects, sample storage time has not been found to influence the parameters measured for up to 4 hours after blood sampling. Store citrated blood at room temperature, NOT at +2 to +8 °C (6). Before analysis, bring citrated blood samples to 37 °C and mix carefully immediately before use 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 FIBTEM test may also be caused by the effect off anticoagulants such as hirudin. The effect of oral anti-coagulants (cumarin) has a secondary influence on the results compared with thromboplastin time. Abnormal fib-TEM 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. Fib-TEM is not designed to be used with native blood.
**Quality Control:**
It is technically impossible to make a stable control with functional thrombocytes. Since fib-TEM® is used together with ex-TEM®, the 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 ranges were obtained for fib-TEM® using reference group samples from Central European adults (hospital staff, blood donors) in three medical centres:(n=148): CT 43-75 s, MCF 9-25 mm, A10 7-23 mm, A20 8-24 mm.
These values should only be used for guidance and must be viewed with caution! They are tentative and may vary from laboratory to laboratory, depending on the collection of blood samples and other pre-analytical factors. Confirmation of these values by a laboratory-/hospital-specific reference group is recommended. .
Platelet function is evaluated by comparing the assays using ex-TEM® and fib-TEM®. The difference in MCF between ex-TEM® and fib-TEM® was 34-54 mm in the samples examined.
**Pathological Results:**
With fib-TEM® maximum clot firmness (MCF) of at least 9 mm should be obtained. Lower values indicate fibrin deficiency and / or a fibrin-polymerisation disturbance.
For conditions under which pathological results in the EXTEM test were obtained, please refer to the ex-TEM® package insert.
**Research applications:**
Information regarding the use of fib-TEM® reagent in conjunction with native blood or in conjunction with hep-TEM® for research purposes can be obtained from Pentapharm GmbH
**Warnings:** For In Vitro Diagnostic Use Only
**Precautions:**
Human blood should be handled with care, following the precautions recommended for potentially infectious substances (7).

## Procedure (FIBTEM assay):

A. Mix the reagents carefully before use. Return any sediment which has formed, **especially in the case of ex-TEM®** carefully into a suspension again. Bring all reagents up to room temperature before use (approx. 15 minutes).
B. Preparation of a citrated blood sample as recommended. Preheat the citrated blood to measuring temperature.
C. NOTE: Follow the ROTEM® user manual for 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 onto 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 fib-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 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 command.

## Performance Data:

**Precision:**

| In Series | CT CV (%) | CFT CV (%) | α-angle CV (%) | A10 CV (%) | MCF CV (%) |
|---|---|---|---|---|---|
| A10-A30;MCF >10 mm | <10*) | **) | **) | <10 | <10 |
| A10-A30; MCF <10 mm | <10*) | **) | **) | <20 | <20 |

*) Precision often reduced due to CT values extended through haemodilution or anticoagulants.
**)CV not determined, as the amplitude(A)-values MCF for the most part did not reach 20 mm.

15 measurements on one single channel of a device or 8-fold assays on two devices.
Blood samples from healthy donors and patients were used for the assays.

**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) the CT mostly reached values around 500-900 sec or the sample becomes incoagulable (CT>15 min).
When using ex-TEM® as an activator the CT is unaffected up to a heparin concentration of 5 U/ml UFH in whole blood (corresponding to 10 U/ml in the plasma)

**Bibliography:**


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(3) Evaluation of the contribution of platelets to clot strength by thromboelastography in rabbits: the role of tissue factor and cytochalasin D. : Vance G, Nielsen VG, Geary BT, Baird MS. Anesth Analg 2000 91: 35-39.
(4) Blutgerinnungsstudien mit der Thromboelastographie, einem neuen Untersuchungsverfahren. Hartert, H.: Klin. Wochenschrift 1948; 26: 577-583
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(6) NCCLS Document H21-A2. Collection, transport, and processing of blood specimens for coagulation testing and performance of coagulation assays, 3rd ed.Approved Guideline 1998
(7) 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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