# Instructions for use – IN RUO assay
# For research use only

REF 1030GL – IN RUO

## Intended purpose

**The IN RUO is a research use only assay for viscoelastometry that allows the assessment of coagulation activation, clot formation and clot stability in whole blood following recalcification and coagulation activation by ellagic acid.**

## Principle of the assay

In the IN assay blood coagulation is activated using ellagic acid and calcium chloride. Ellagic acid stimulates the intrinsic coagulation cascade by the so-called contact phase. This results to an activation of the coagulation factor XII to XIIa, which via the factors XI, IX, VIII, X and V leads to the activation of prothrombin to thrombin. Thrombin converts fibrinogen to fibrin and activates platelets, which leads to the clotting of the sample. This is detected by the clotting time (CT) of the test.

When unfractionated heparin in the sample is present, it forms complexes with antithrombin and inhibits formed FXa and thrombin, as well as the generation of these activated clotting factors. Therefore, the clotting time (CT) of the in assay is prolonged, that allows detecting the effect of the unfractionated heparin in this test.

The use of ellagic acid- activated viscoelastometry for the detection of unfractionated heparin was reported by Mittermayr et al [1] and is a widely used standard procedure [2].

## Materials provided

10 sealed single-use pouches containing one pipet tip with reagent each, providing a dry chemistry reagent composed of ellagic acid, calcium chloride, buffer and stabilizers. Each pouch further contains one desiccant bag.

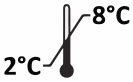
## Additional materials and devices required

- Viscoelastometry analyzer and receptacles (Cups & Pins)
- Electronic pipette for 340 µl with 3 sec aspiration / dispensing cycles
- Blood collection tube (3.2% sodium citrate) for coagulation testing

## Reagent preparation

The reagent is ready to use.

## Storage and stability




Store at +2 to +8 °C. The unopened reagent tips are stable until the expiration date stated on the pouch label. Unopened pouches may be stored at room temperature for up to 1 month. Opened pouches are for immediate use within 1 minute after opening the pouch.

## Warnings and precautions

For professional use by trained personnel.

Do not use tips from defective pouches or from pouches missing the desiccant pack.

Intended for single use - do not reuse.

Human blood samples should be handled with care, following general precautions recommended for bio- hazardous materials [3].

General precautions (e.g., wear gloves and minimize skin exposure to specimen and reagents) should be followed when handling all materials. Dispose of waste according to local regulations. A material safety data sheet is available upon request.

## Sample collection

Collect the sample according to the recommended procedures [4][5]. Samples should be analyzed within 3 hours from blood collection. Always ensure blood collection tubes are filled to the indicated fill volume to avoid excessive citrate levels.

## Test procedure

1. Check the expiry date of the device. Expiry date format is yyyy-mm-dd. Do not use expired product.
2. Allow the reagent tip pouch to reach room temperature.
3. If the sample is cold (< 22°C) it is advised to allow the sample to warm up for 5 min on the heated position of the viscoelastometry analyzer. In evaluations on the effect of pre-warming blood tubes which had room temperature little to no effect was observed vs. tubes which were not pre-warmed.
4. Create the test in the software of the viscoelastometry analyzer according to the analyzer manual.
5. Place the Cup and Pin into the analyzer according to the analyzer manual.
6. Tear open the reagent tip pouch, attach the reagent tip to the electronic pipette and aspirate 340 µl sample from the blood tube.
7. Dispense the blood sample into the Cup.

8. Aspirate and dispense the sample once again to facilitate thorough mixing of the reagents with the blood sample. Ensure sample pipetting is performed without interruption of the process.
9. Start the test as described in the analyzer manual.
10. The test will stop, or you can stop the test as described in the analyzer manual.
11. Remove the Cup & Pin and dispose according to local regulations.

## Quality control

Plasma-based quality control materials can be used to confirm the stability of test results determined with the IN assay over time.

## Result interpretation and expected values

The interpretation of the IN assay is performed using the clotting time (CT) for the evaluation of the coagulation activation, using the A5, A10, A20 parameter for the evaluation of the clot formation and by the maximum lysis (ML) for the assessment of clot stability.

The presence of unfractionated heparin or factor deficiencies of the intrinsic pathway can lead to prolongations of the clotting time (CT), if they result to delayed generation of free thrombin in the sample. An impaired thrombin generation can also lead to a weaker clot formation. An impaired clot stability is detected by an increased maximum lysis (ML).

The clot curve determined during the analysis should be smooth and not noisy. Repeat measurements with irregular curves.

## Manufacturer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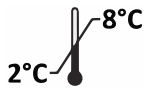
## Symbols

| Symbol | Meaning |
|---|---|
|  | Manufacturer |
|   | Batch code |
|   | Country of manufacture |

| Symbol | Meaning |
|---|---|
|  | Use-by date |
|   | Catalogue number |
|  | Do not use if package is damaged and consult instructions for use |

| Symbol | Meaning |
|---|---|
|   | Temperature limit |
|  | Consult instructions for use or electronic instructions for use |

| Symbol | Meaning |
|---|---|
|  | Do not re-use |
|   | Contains sufficient for <n> tests |

## References

[1] Mittermayr M, Margreiter J, Velik-Salchner C, Klingler A, Streif W, Fries D, Innerhofer P. Effects of protamine and heparin can be detected and easily differentiated by modified thrombelastography (Rotem): an in vitro study. Br J Anaesth. 2005 Sep;95(3):310-6

[2] Scala E, Marcucci C. Massive Hemorrhage: The Role of Whole Blood Viscoelastic Assays. Hamostaseologie. 2020 Nov;40(4):515-523

[3] Biosafety in microbiological and biomedical laboratories; U.S. Department of Health and Human Services, Washington, 5th Edition

[4] CLSI/NCCLS H03-A6; Procedures for the collection of diagnostic blood specimens by venipuncture

[5] CLSI H21-A5 Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays

## Version history of these instructions for use

| Date | Version | Change description |
|---|---|---|
| 2025-03-26 | 1 | Initial version |