GFC-Test

REF CK093K

R1 3 x 2 mL, R2 3 x 2 mL

Determination of the Global Fibrinolysis Capacity
in human citrated plasma.

HYPHEN BioMed

Vertrieb und Support:
CoaChrom Diagnostica GmbH
www.coachrom.com | info@coachrom.com
Tel. +43-1-236 222 1 | Fax +43-1-236 222 111
Kostenfreie Nummern für Deutschland:
Tel. 0800-24 66 33-0 | Fax 0800-24 66 33-3

INTENDED USE:

The GFC-Test kit is a method for qualitative determination of clot lysis time in human citrated plasma.

SUMMARY AND EXPLANATION:

Technical:

The Global Fibrinolytic Capacity test permits to determine the clot lysis time after addition of thrombin and calcium in the presence of an exogenous supply of tissue plasminogen activator (tPA), at a limited and constant concentration, and silica.

PRINCIPLE:

In a first step, tPA with silica R1 is introduced into the tested plasma. Then human thrombin R2, in the presence of calcium, is added, which triggers coagulation, subsequently followed by clot dissolution (due to fibrinolysis enhanced by the presence of tPA). The absorbance is then followed at exactly 940nm until clot lysis is complete. The detection of the inflection point of the lysis curve corresponds to the lysis time[1,2].

Silica + Thrombin + Ca^{2+} + plasma ⟶ Clot

Clot + exogenous tPA ⟶ Lysed Clot

REAGENTS:

R1 **tPA with silica**. Tissue Plasminogen Activator (tPA) with silica, lyophilized form. Contains BSA.
3 vials of 2 mL.

R2 **Thrombin reagent**. Human thrombin at 4 NIH/mL, lyophilized form. Contains BSA and Calcium.
3 vials of 2 mL.

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* use is intended for professional use in the laboratory.

REAGENT PREPARATION:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

R1 R2 Reconstitute the contents of each vial with exactly **2 mL of distilled water**.
Shake vigorously until complete dissolution while avoiding formation of foam and allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R1 R2 Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

- **24 hours** at 2-8°C.
- **8 hours** at room temperature (18-25°C).
- **1 month** frozen at -20°C or less*

*Thaw only once, as rapidly as possible at 37°C and use immediately. The stability of the thawed reagent should be checked under laboratory work conditions.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Specific controls with known titration, such as:

Product Name	Reference
GFC Control Plasmas	SC104K

Materials:

- Instrument and software for the determination of clot lysis time or incubated spectrophotometer.
- Cuvette fitted to the instrument used.
- Stopwatch, Calibrated pipettes.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.
Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[3] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[4,5,6].

PROCEDURE:

Assay method:

1. Samples are tested undiluted.
2. Introduce in a cuvette at 37°C:

	Cuvette
Sample or control	100 µL
R1 **tPA with silica**, homogenized before use	100 µL
Mix and incubate at 37°C, for about 1 minute then introduce :	
R2 **Thrombin reagent**	100 µL
Mix by vortexing and start continuous reading at 940nm. Stop the reading when lysis is complete.	

If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

Indicative:

- Normal plasmas lysis time is included in 30 to 60 minutes, with a mean value around 40 minutes.
- A lysis time shorter than 30 minutes correspond to a hyperfibrinolysis.
- A lysis time longer than 60 minutes correspond to a fibrinolysis default and this especially since the time is long.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- The absence of coagulation that could be caused by inhibitors, anticoagulant or fibrinogen deficiency, leads to erroneous results. Always visually confirm the presence of a coagulation wave on the curve obtained, no later than 10 minutes after the introduction of R2. In case of absence of coagulation, the results should not be considered.

PERFORMANCES:

- Performance studies were conducted internally on Instrument for the determination of clot lysis time. The following results were obtained:

Control	Inter assays			
	n	Mean	CV%	SD
Hyperfibrinolysis Control	6	16	3.5	0.5
Normal GFC Control	6	39	3.5	1.4
Hypofibrinolysis Control	6	77	2.8	2.1

REFERENCES:

1. Rijken DC *et al.* Development of a new test for the global fibrinolytic capacity in whole blood. J Thromb Haemost. 2008.
2. Zouaoui Boudjeltia K *et al.* A new device for measurement of fibrin clot lysis: application to the Euglobulin Clot Lysis Time. BMC Biotechnology. 2002.
3. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
4. Mauge L. and Alhenc-Gelas M. Stabilité pré-analytique des paramètres de la coagulation: revue des données disponibles. Ann Biol Clin. 2014.
5. Woodhams B. *et al.* Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Changes compared to the previous version.

HYPHEN BioMed
155 rue d'Eragny, 95000 Neuville-sur-Oise, France