CEPHEN 1

REF CK511K

R1 6 x 1 mL

Kit for determination of aPTT clotting time
with ready to use liquid reagents

Not for Sale in the US

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INTENDED USE:

The CEPHEN 1 kit is proposed for determination of activated Partial Thromboplastin Time (aPTT) on citrated human plasma, using a manual, semi automated or automated clotting method with liquid reagents ready to use. This reagent has a low sensitivity to Lupus Anticoagulant and can be used for an exploration of Lupus Anticoagulant.

SUMMARY AND EXPLANATION:

A prolonged aPTT can result from[1]:
Presence of "anticoagulant" activity induced by therapy (Heparin, Hirudin, Argatroban®, Angiox®, AVK…).
Factor deficiencies: II, V, X (<5 to 10%), VIII:C, IX, XI, XII (<20%), including high molecular weight kininogen (<5%).
Abnormalities or acquired deficiencies due to an excessive consumption of the coagulation factors, hepatic disorders…
Coagulation inhibitors such as Lupus Anticoagulant or auto-antibodies to coagulation factors.
However, the CEPHEN reagent sensitivity to lupus anticoagulants is intentionally less sensitive than most other routine aPTT reagents.

PRINCIPLE:

Measurement of the plasma recalcification time in presence of the standardized aPTT (Activated Partial Thromboplastin Time) reagent (cephalin and activator), on human citrated plasma, to explore the activity of the intrinsic pathway coagulation factors (II, V, VIII:C, IX, X, XI, XII).
This reagent has intentionally less sensitivity to lupus anticoagulants than routine reagents. Otherwise it is similar to CEPHEN LS reagent.

REAGENTS:

R1 **aPTT**, liquid form, ready to use.
6 vials of 1mL.

Reagent contains small amounts of sodium azide (0.9 g/L), see WARNINGS AND PRECAUTIONS.

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- The reagent may be opalescent, with possible presence of whitish to greyish siliceous sediments disappear after shaking.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface. Evaporation reduces the reagent's stability in the analyzer.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies, conducted over a 3-week period at 30°C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- For *in vitro* diagnostic use.

REAGENT PREPARATION AND STABILITY:

R1 **Reagent 1**: **aPTT**
Ready to use. Allow to stabilize for 30 minutes at room temperature (18-25°C), before use.
Homogenize the reagent prior to use.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:

- **3 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Do not freeze.**

STORAGE CONDITIONS:

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.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- $CaCl_2$ 0.025 M (AR001A/K).
- Specific controls plasma for aPTT and LA, such as:

Product Name	Reference
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301
LA Control Plasma	SC081K / SC082K / SC083K

Materials:

- Water-bath, semi-automatic or automatic instrument for clotting assays.
- Stopwatch, Calibrated pipettes.

SPECIMEN COLLECTION AND PREPARATION:

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5 guidelines for further information concerning specimen collection, handling and storage).

- Specimens:

Human plasma obtained from anticoagulated blood (trisodium citrate).

- Collection:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M) by clean venipuncture. Discard the first tube.

- Centrifugation:

Within 2 hours, use a laboratory-validated method to obtain platelet-poor plasma, for example at least 15 minutes at 2500 g at room temperature (18-25°C) and allow the plasma to settle in a plastic tube.

- Plasma storage[2-3]:
  - 4 hours at room temperature (18-25°C).
  - 1 month at -20°C.
  - 18 months at -70°C.

Frozen plasma specimens should be thawed rapidly at 37°C, then shaken thoroughly and tested immediately. Resuspend any precipitate by shaking vigorously immediately after thawing and before use.

PROCEDURE:

The kit is a clotting method, automated or manual (endpoint) methods. Perform the test at 37°C and the clotting time, triggered by addition of 0.025M Calcium Chloride, is measured.

Automated methods:
Applications for the various analyzers are available on request. **See the specific application and specific precautions for each analyzer.**

Assay method:
A mechanical coagulation indicator, such as a metal ball or index, or balancing, is used for detecting clotting.
The plasma must be tested **undiluted**.
In a plastic tube or in microassay well, incubated at 37°C, introduce:

	Volume
Specimen or control undiluted	100 µL
CEPHEN reagent	100 µL
Mix and incubate at 37°C, exactly for **3 minutes**, then introduce (Starting the stop-watch):	
0.025 M Calcium Chloride **preincubated at 37°C**	100 µL
Record the exact clotting time, in seconds (stop of the metal ball or index, or coagulation detected by clot formation…)	

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, at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptable range for the method.
Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

- Clotting time can slightly vary according to the type of citrated anticoagulant used.
- The results should be interpreted according to the patient's clinical and biological condition

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully. The laboratory is responsible for validating any changes made to these instructions for use.
- 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.
- Any plasma displaying a coagulum or showing signs of contamination must be rejected.
- Various drugs or therapies can affect aPTT results. An additional investigation should be conducted to determine the origin of each unexpected or abnormal result.
- A "repeat" clotting time for a sample even with the same reagent lot can vary slightly according to the instrument used, and the clot detection mode and instrument setting (clot detection sensitivity).
  Each laboratory should establish and validate its own usual range, mean and standard deviation, in its specific test conditions.
  In the same way, many variables (e.g.: different sources of heparin) can affect the results obtained: each laboratory should establish its own acceptable ranges.
- The 3 min. incubation time must be adhered to consistently. If this incubation time must be changed for the needs of an instrument application (for example 4 min.), it must be the same for all the tests performed.
- The reagent offers a good sensitivity for a prekallicrein deficiency <1%, but no sensitivity for concentrations >5%.
- Using the KC10 instrument, no significant interference was noticed up to <0.25 mg/ml bilirubin, or up to <5 mg/ml haemoglobin added to plasma.
- Reagent is very sensitive to Unfractionated Heparin (UFH) in plasma, and clotting time prolongation is significant from 0.1 IU/ml. This sensitivity is lower for Low Molecular Weight Heparin (LMWH).
- Heparin sensitivity is variable for the various aPTT reagents marketed, is variable from reagent to reagent, and for a same reagent, it can present slight variations from lot to lot for a same reagent. Heparin sensitivity must be checked by the laboratory in the actual conditions of testing, and for the lot used. The same plasma heparin concentration can produce variable prolongations of the aPTT, and of the clotting time ratio Patient/Normal Control; especially for patients in intensive care units or resuscitation.

EXPECTED VALUES:

As an indication, aPTT values obtained on KC10 or STA-R, for normal plasmas, are usually in the range: 28 sec to 37 sec.
aPTT is abnormal if: > 40 seconds.
The obtained aPTT for the patient must be compared with that of the reference normal range for the laboratory.
The clotting times obtained can vary according to the citrated anticoagulant used (0.109 or 0.129M), as well as to the clot detection mode (mechanical or optical). Clotting times are shorter when using 0.109M citrate, and an optical detection mode.

The reagent can be used for an exploration of Lupus anti-coagulant[4-5-6].
LA are absent from normal human plasmas.
As an indication, for normal plasma, CT value is usually expected **< 45 sec**, and normalized ratio **< 1.20.**

However, each laboratory has to determine its own normal range.

PERFORMANCE:

- As an example, the "usual" aPTT range has been determined for citrated normal human plasmas using:

Lot 070622B	KC10	STA-R	ACL 700 (research software) (optical mode)
N	30	50	30
M (Mean aPTT, sec)	28.8	31.4	25.1
SD (sec)	2.01	2.66	1.77
M ± 2SD (sec)	24.8-32.8	26.1-36.7	21.6-28.6
Min-Max (sec)	25.6-33.5	27.1-39.2	22.2-30.0

- The CEPHEN reagent clotting times obtained on normal plasmas show a good consistency with STA-PTT® reagent, performed on STA-R instrument, and with CK Prest® reagent performed on KC10 instrument:

	CEPHEN 070622B (STA-R)	STA-PTT® (STA-R)	CK Prest® (KC10)
Mean aPTT (N=30 normals)(sec)	33.2	32.4	32.7
Mean ± 2SD	28.1-38.3	28.3-36.5	28.3-37.1

- Good sensitivity to hirudin, using the KC10 instrument, from 0.1 to 0.2 µg/mL in plasma.

REFERENCES:

1. "Hémorragies et thromboses – Du diagnostic au traitement", M.M. Samama et coll., Abrégés, Masson, 2004.
2. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
3. Woodhams B. *et al.* Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.
4. WHO Reference Panel 1st International Reference Panel for Lupus Anticoagulant NIBSC code: 13/172, 2015.
5. Kumano O. et al. Use of a lupus anticoagulant-resistant routine APTT reagent as a convenient confirmatory test. ISTH 2016 Abstract.
6. H60-A Document: "Laboratory Testing for the Lupus Anticoagulant; Approved Guideline". 2014.

SYMBOLS:

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

Changes compared to the previous version.