CEPHEN™

CE

IVD

REF	CK511K	R	6 x 1 mL
REF	CK512K	R	6 x 2.5 mL
REF	CK515K	R	8 x 5 mL
REF	CK515L	R	12 x 5 mL

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

HYPHEN BioMed
155 rue d'Eragny, 95000 Neuville-sur-Oise, France
Tél : +33 (0)1 34 40 65 10
Fax : +33 (0)1 34 48 72 36
www.hyphen-biomed.com
info@hyphen-biomed.com

English, last revision: 02-2022

INTENDED USE:

The CEPHEN™ kits are proposed for the *in vitro* quantitative determination of activated Partial Thromboplastin Time (aPTT) on citrated human plasma, using manual or automated clotting method with liquid reagents ready to use (LRT, Liquid reagent Technology). This reagent, with a moderate sensitivity to Lupus Anticoagulant (LA), can be used in combination with a highly sensitive reagent (CEPHEN™ LS) for the exploration of LA.

SUMMARY AND EXPLANATION:

Technical:

Measurement of the plasma recalcification time, in presence of the standardized aPTT reagent (phospholipids and activator), on human citrated plasma, as a global screening test to explore the activity of the coagulation Factors (II, V, X, VIII: C, IX, XI, XII) and Fibrinogen.[1]

Clinical:

The aPTT is a screening test to assess:[1-4]

- Some anticoagulant therapies.
- Abnormalitie of intrinsic coagulation pathway factors.
- Abnormalities or acquired deficiencies due to an excessive consumption of the coagulation factors, hepatic disorders…
- Coagulation inhibitors such as LA or auto-antibodies against coagulation factors.

However, the CEPHEN™ reagent sensitivity to LA is intentionally less sensitive than most other routine aPTT reagents.

PRINCIPLE:

CEPHEN™ is an activated Partial Thromboplastin Time (aPTT) reagent.

Activation of intrinsic pathway on citrated plasma is induced by activator (micronized silica) and vegetable soybean phospholipids, and the clotting time (CT) is measured in presence of calcium.[1]

REAGENTS:

R **aPTT**, liquid form.

REF **CK511K** ➔ **6 vials of 1 mL**
REF **CK512K** ➔ **6 vials of 2.5 mL**
REF **CK515K** ➔ **8 vials of 5 mL**
REF **CK515L** ➔ **12 vials of 5 mL**

WARNINGS AND PRECAUTIONS:

- 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* diagnostic use is intended for professional use in the laboratory.

REAGENT PREPARATION:

R Reagent is ready to use; homogenize and load it directly on the analyzer following application guide instruction.

For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

The reagent can be opalescent, with possible presence of whitish to greyish siliceous sediments, which disappear after shaking.

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.

R Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

- **3 months** at 2-8°C.
- **7 days** at room temperature (18-25°C).
- **Do not freeze.**
- **Stability on board of the analyzer: see the specific application.**

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- $CaCl_2$ 0.025M (AR001B/AR001K/AR001L).
- Specific controls plasma for aPTT and LA, such as:

Product Name	Reference
BIOPHEN™ Normal Control Plasma	223201
BIOPHEN™ Abnormal Control Plasma	223301
EASYPLASMA™ Control Set	225601
LA Control Plasma	SC081K / SC082K / SC083K

Also refer to the specific application guide of the analyzer used.

Materials:

- Water-bath, semi-automatic or automatic instrument for clotting assays.
- Stopwatch; Calibrated pipettes; silicon glass or plastic test tubes.

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[5] guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references[5-7].

PROCEDURE:

The kit is to be used with manual or automated methods. Perform the test at **37°C** and measure clotting time, triggered by addition of 0.025M Calcium Chloride.

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

Assay method:

1. Reconstitute, if necessary, the controls as indicated in the specific instructions.
2. Plasma should be tested **undiluted.**
3. Introduce into a reaction cuvette, silicon glass or plastic test tube incubated at **37°C**:

	Volume
Specimen or controls undiluted	100 µL
R **aPTT**	100 µL
Mix and incubate at **37°C**, exactly for **3 minutes**, then introduce (Starting the stop-watch):	
0.025M 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 different from that specified above is required for the method used, the volume ratios must be strictly observed to guarantee assay performance. The user is responsible for validating any change and impact on 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, to validate the test.
Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

- The obtained CT for the sample must be compared with that of the reference normal range for the laboratory (refer to current local recommendations).
- Results can be reported as a ratio:
  APTT ratio = Sample (CT, sec) / Mean of normals (CT, sec).
- The reagent must be used in combination with the high sensitivity reagent, CEPHEN™ LS (CK521K/CK522K), for exploration of LA[2-7].
- 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.
- 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.
- 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" CT for a sample even with the same reagent lot can vary slightly according to the instrument used and the clot detection mode.
- For optimal heparin sensitivity, the tested plasma should be collected and processed without activation and release of platelet alpha granules, which contain PF4, a heparin inhibitor.[5,6]
- Heparin sensitivity can present slight variations from lot to lot for a same reagent. The same anticoagulant plasmatic concentration (heparin) can produce variable prolongations of the aPTT, in particular for patients in intensive care units or resuscitation[8-9].
- This reagent has a moderate sensitivity to LA.
- The reagent offers a good sensitivity for a prekallicrein deficiency <1%, but no sensitivity for concentrations >5%.

EXPECTED VALUES:

As an example, for one lot, the reference range established on healthy adult subjects (n=120) using Sysmex CS-5100 (Central 90%, 95th percentile) was measured between 24 and 35 sec. Each laboratory has to determine its own usual ranges (normal range, heparin sensitivity…) for each combination of lot and instrument used.

PERFORMANCE:

- Reagent is sensitive to low concentrations of plasmatic heparin (from 0.1 IU/mL for Unfractionated Heparin).
- Performance studies were conducted internally on Sysmex CS-5100. Performance was assessed using laboratory controls over a 5-day period, 2 series per day and 2 repetitions within each series for a control level. The following results were obtained:

Control	Intra assay				Inter assays			
	n	Mean	CV%	SD	n	Mean	CV%	SD
Control 1	40	29.5	0.3	0.1	20	29.6	1.4	0.4
Control 2	40	58.1	0.3	0.2	20	58.5	0.9	0.5

- Correlation with reference method (Dade Actin FS APTT Reagent on Sysmex CS-5100 on aPTT seconds) :
  n = 150 y = 1.21x -2.64 r = 0.958
- Interferences:
  No interference, on the analyzer Sysmex CS-5100 was observed with the molecules and up to following concentrations:

Intralipids (mg/dL)	Hemoglobin (mg/dL)	Bilirubin (F/C) (mg/dL)	Apixaban (ng/mL)
1000	1000	30	50

Also refer to the specific application guide of the analyzer used.

REFERENCES:

1. Kamal AH. *et al.* How to interpret and pursue an abnormal prothrombin time activated partial thromboplastin time, and bleeding time in adults. Mayo Clin Proc. 2007.
2. Kumano O. *et al.* Use of a lupus anticoagulant-resistant routine APTT reagent as a convenient confirmatory test. ISTH 2016 Abstract.
3. H60-A Document: "Laboratory Testing for the Lupus Anticoagulant; Approved Guideline". 2014.
4. Kumano O. *et al.* Paired APTTs of low and high lupus anticoagulant sensitivity permit distinction from other abnormalities and achieve good lupus anticoagulant detection rates in conjunction with dRVVT. International Journal of Laboratory Hematology. 2018.
5. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
6. Woodhams B. *et al.* Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.
7. WHO Reference Panel 1st International Reference Panel for Lupus Anticoagulant NIBSC code: 13/172, 2015.
8. Van Roessel S. et al. Accuracy of aPTT monitoring in critically ill patient treated with unfractionated heparin. The Journal of Medicine. 2014.
9. Gouin-Thibaut I. *et al.* Monitoring unfractionated heparin with APTT: A French collaborative study comparing sensitivity to heparin of 15 APTT reagents. Thrombosis Research 129. 2012.

SYMBOLS:

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

Changes compared to the previous version.