

CEPHEN™

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

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

FOR RESEARCH USE ONLY.

DO NOT USE IN DIAGNOSTIC PROCEDURES.

English, last revision: 10-2020

INTENDED USE:

The CEPHEN™ kits are proposed for the *in vitro* qualitative 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.

This kit is for research use only and must not be used for patient diagnosis or treatment.

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.

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.

REAGENTS:

R aPTT, liquid form. Contains small amounts of sodium azide (0.8 g/L).

REF	CK511K-RUO →	6 vials of 1 mL
REF	CK512K-RUO →	6 vials of 2.5 mL
REF	CK515K-RUO →	8 vials of 5 mL
REF	CK515L-RUO →	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.
- In contact with lead or copper pipes, sodium azide can generate explosive compounds.
- 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:

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.
- CaCl₂ 0.025M (AR001A/K-RUO).
- Specific controls plasma for aPTT and LA, such as:

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

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-A5¹ guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references^{1,2}.

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-RUO/CK522K-RUO), for exploration of LA.

The results obtained should be for research use only and must not be used for patient diagnosis or treatment.

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 substances 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, the clot detection mode (mechanical or optical) and instrument setting (clot detection sensitivity).
- 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.
- 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.
- This reagent has a moderate sensitivity to LA.
- The reagent offers a good sensitivity for prekallikrein <1%, but no sensitivity for concentrations >5%.

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. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008
2. 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.