

**LUPAKCT****Kaolin clotting time reagent for the detection of Lupus anticoagulants****SUMMARY**

Lupus anticoagulants (LA) screens are frequently investigated in thrombophilic patients or women with fetal loss. The LA antibodies recognize the Prothrombin-phospholipid complex (Human), thereby inhibiting the phospholipid dependant coagulation reactions. The kaolin clotting time (KCT) has often been regarded as the most sensitive test for the detection of circulating anticoagulants. The KCT detects all class of inhibitors, including those directed against factor VIII and contact activation as well as heparin. To overcome this issue more specific tests have been developed such as the dilute Russell's viper venom test (DRVVT) available as LADS (Ref: 10671010) from Tulip, which bypass most of the upper stages of clotting mechanism. However in many clinical situations like detection of LA in pregnancy it is often desirable to use a broad screening method such as Lupakct (kaolin clotting time) reagent.

**PRESENTATION**

<b>REF</b>	10672050	10672020
<b>R1</b>	5 ml	2 ml
<b>R2</b>	5 ml	2 ml
Pack insert	1	1

**REAGENTS**

1. Lupakct kaolin clotting time reagent (R1): contains stabilized kaolin suspension in buffers and preservatives.
2. Calcium chloride (R2): 0.025 M calcium chloride solution

**REAGENT STORAGE AND STABILITY**

Store the reagent at 2-8°C. DO NOT FREEZE.

The shelf life of the reagent is as per the expiry date mentioned on the reagent vial labels. The uncontaminated reagent is stable for 1 year at 2-8°C.

**PRINCIPLE**

The kaolin clotting time is essentially an activated partial thromboplastin time test without added phospholipids. The latter is believed to be the main target of antiphospholipid antibodies which are often associated with lupus anticoagulants and which therefore prolong the test. If the test is performed on a range of mixtures of normal and patient's plasma, different patterns of response are obtained indicating the presence of lupus anticoagulants, deficiencies of one or more of the coagulation factors or the lupus cofactor effect.

**NOTE**

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. Kaolin clotting time reagent is not from human source hence contamination due to HBsAg, HCV and HIV is practically excluded.
3. Reagent contains 0.01% Thimerosal as preservative.
4. It is very important that clean and dry micropipette tips be used to dispense the reagent.
5. Avoid exposure of the reagent to elevated temperature and contamination. Immediately replace cap after use and store at recommended temperatures only.
6. Do not use damaged or leaking reagents.

**SAMPLE COLLECTION AND PREPARATION**

No special preparation of the patient is required prior to sample collection by approved techniques. Withdraw blood without undue venous stasis and without frothing into a plastic syringe fitted with a short needle of 19 to 20 SWG. The veinpuncture must be a 'clean' one and, if there is any difficulty, take a new syringe and needle and try another vein. Transfer the blood into tubes, after detaching the needle from the syringe.

Mix exactly nine parts of freshly collected blood with one part of tri-sodium citrate (0.11 mol/l, 3.2%). Centrifuge immediately for 20 minutes at 3000 rpm (approximately 2000 g) and transfer the plasma into a clean test tube. Plasma must be tested within three hours of blood collection.

**ADDITIONAL MATERIAL REQUIRED**

12 x 75 mm test tubes (plastic tubes are preferred), pipettes, Stopwatch, Water bath or heating block at 37°C, Fresh normal plasmas.

## TEST PROCEDURE

### Manual Method

1. Bring reagents to room temperature. Before use, the reagent should be mixed well by gentle swirling. Do not shake.
2. Aspirate from the reagent vial enough reagent for the immediate testing requirement in a thoroughly clean and dry test tube. Separate test tubes containing Lupakct kaolin clotting time reagent and Lupakct Calcium Chloride Solution should be brought to 37°C. (Depending on volume, approximately 5 to 10 minutes are required). Do not incubate the test plasma.
3. To a 12 x 75 mm test tube, add 100 µl test plasma and 100 µl Lupakct kaolin clotting time reagent. Shake the tube briefly to mix the reagent and plasma and place the tube at 37°C for 3 to 5 minutes.
4. Following incubation period, add forcibly 100 µl of prewarmed calcium chloride into the plasma and Lupakct kaolin clotting time reagent mixture, simultaneously start a stopwatch.
5. Gently tilt the tube back and forth and stop the stopwatch as soon as the first fibrin strand is visible and the gel / clot formation begins. Record the time in 'seconds'.
6. Repeat steps 3-5 for a duplicate test on the same sample.
7. Find the average from the duplicate test values. This is the kaolin clotting time of the patient plasma .
8. Similarly repeat steps 3-5 twice, and record duplicate values using normal plasma in place of test plasma and find the average of duplicate values. This is the KCT of the normal plasma.

### Mixing studies

As the KCT is sensitive to many kinds of clotting abnormalities to make it more specific for circulating inhibitors the KCT should be carried out on mixtures of patient and normal plasma.

1. Mix 500 µl patient plasma with 500 µl normal plasma to obtain 50: 50 mix in one test tube.
2. Mix 200 µl patient plasma with 800 µl normal plasma to obtain a 20: 80 mix in another test tube.
3. Repeat steps 3-7 and obtain the KCT time for each of the 50:50 mix and 20:80 mix.

If a coagulation instrument is being used to perform the tests, the instrument manufacturers instructions must be strictly adhered to. Any instrument protocol for performing an APTT test can be used for determining the KCT with Lupakct simply by substituting the APTT reagent with Lupakct kaolin clotting time reagent.

### INTERPRETATION OF RESULTS

KCT results can be expressed as:

1. KCT in seconds
2. KCT ratio ( $\Delta$  KCT) using a 20:80 mixture of Patient: normal plasma

$$\Delta \text{ KCT} = \frac{[\text{KCT (20:80 mix)} - \text{KCT (Normal)}]}{\text{KCT (normal)}}$$

3. Rosner Index (RI) using a 50:50 mixture of patient: normal plasma

$$\text{RI} = \frac{\text{KCT (50:50 mix)} - \text{KCT (normal)}}{\text{KCT (Patient)}}$$

An abnormality may be suspected when the test KCT is more than the normal range (60 - 120 seconds). This abnormality is more likely to be lupus anticoagulant if:

1. KCT is more than 130 seconds
2.  $\Delta$  KCT > 0.10
3. RI > 0.15

As the clotting time obtained between instruments with different measuring principles and also between different populations may vary, each laboratory should establish its own normal range.

### REMARKS

1. A control KCT of < 60 seconds may indicate contamination of the normal (control) plasma with phospholipid.
2. Lupakct is not recommended for patients undergoing anticoagulant therapy with heparin.
3. The performance of Lupakct must be validated using normal and known LA positive plasmas.
4. Homogenisation of Lupakct Kaolin Clotting Time Reagent suspension is important to achieve accurate and consistent results.

**PERFORMANCE CHARACTERISTICS**

Precision studies were performed using normal plasma pool sample, LA high control (n=15) on Hemostar XF coagulometer. Inter/ Intra assay precision

Samples	Mean	SD	CV (%)
Normal plasma control	74.9	3.4	4.5
LA high control	271	10.1	3.7

**SPECIFICITY**

Specificity studies were performed in known LA high control and LA low control, 50 normal plasma samples, and Factor deficient plasmas.

















**WARRANTY**

The product is designed to perform as described on the label and the package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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6. Data on file: Tulip Diagnostics (P) Ltd., India.

### SYMBOL KEYS

	Temperature limitation		Manufacturer		Contains sufficient for <n> tests		Production Site
	Use by		Consult Instructions for use		Kaolin Clotting Time Reagent		
	Date of Manufacture		Catalogue Number		Calcium Chloride		
	Batch Number		<i>In vitro</i> Diagnostic Device		Description of reagent		
	This way up		Authorised Representative in the European Community		KCT reagent for the detection of Lupus Anticoagulants		

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