

# LupoTek DetecTin

## LupoTek CorrecTin

Lupus Anticoagulant Testing Kits



### INTENDED USE

The **LupoTek DetecTin** reagent is intended for use in a Dilute Russell's Viper Venom time test (DRVVT) to screen for the presence of Lupus Anticoagulants (LA) in plasma. The **LupoTek CorrecTin** reagent is a high phospholipid DRVVT reagent for confirmation of lupus anticoagulants. The **LupoTek DetecTin** and **CorrecTin** reagents are intended to be used in conjunction with each other in simplified Dilute Russell's Viper Venom Time tests.

### SUMMARY

Lupus anticoagulants are antiphospholipid autoantibodies targeted against complexes of proteins and negatively charged phospholipids. The target proteins include prothrombin, beta-2-glycoprotein I, annexin V and others. Lupus anticoagulants are a subtype of this group of autoantibodies recognized by coagulation tests. Clinically they are associated with auto-immune disease (1), recurrent fetal loss (2) and unexplained thrombosis, both venous and arterial (3).

Circulating anticoagulants are usually detected by the presence of a prolonged clotting time in global tests (4) such as an activated partial thromboplastin time (APTT) or DRVVT, which does not correct on mixing patient plasma (1:1) with normal plasma. These tests are not specific, and cannot distinguish between a Factor VIII inhibitor, heparin contamination and a true antiphospholipid antibody.

The hallmark characteristic of lupus anticoagulants is their phospholipid dependence, which is best elucidated by using a reagent with a high phospholipid content, which corrects the prolonged clotting time. The Dilute Russell Viper Venom time (DRVVT) is a simple one stage clotting test which can be used with carefully matched low and high phospholipid reagents to detect Lupus Anticoagulants with minimal interference from other types of circulating anticoagulants (5).

### PRINCIPLE

Russell's viper venom will directly activate Factor X without requiring Factor VII. The activated Factor X in conjunction with Factors V, II, calcium ions and phospholipid will generate thrombin which converts fibrinogen to fibrin, producing a clot in the test system.

Because the DRVVT does not involve any intrinsic pathway factors, it is not sensitive to contact factor deficiencies, hemophilia A or B or Factor VIII inhibitors. The **LupoTek DetecTin** is designed as the screening reagent to detect a prolongation of the clotting time, while the **LupoTek CorrecTin** is a high phospholipid reagent which should neutralize the LA and correcting the clotting time to normal, confirming the presence of a Lupus Anticoagulant. Since the **LupoTek DetecTin** test is sensitive to deficiencies of Factors II, V and X, a mixing test utilizing a mixture (1: 1) of patient plasma and normal plasma can help identify a factor deficiency in which the mixing study will correct, from an inhibitor in which the mixing study should not significantly correct towards the normal plasma clotting time.

### REAGENTS

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.**

#### 1. LupoTek DetecTin

**Ingredients:** Russell's viper venom, phospholipids, antiheparin agents, calcium ions, buffers, stabilizers and a green dye (<0.001%). Sodium azide (0.05%) is used as a preservative.

**Preparation for Use:** Reconstitute the vial with **2 mL** distilled water. Mix well, do not shake and leave at room temperature for 10 minutes before use.

**Storage and Stability:** The lyophilized reagent is stable until the expiration date printed on the vial. After reconstitution, the reagent is stable for 48 hours at 2-8° C. The reagent may be stored at -20° C for 1 month. It should be thawed rapidly at 37° C before use.

#### 2. LupoTek CorrecTin

**Ingredients:** Russell's viper venom, high concentrations of phospholipids, anti-heparin agents, calcium ions, buffers, stabilizers and a red dye (<0.001%). Sodium azide (0.05%) as a preservative.

**Preparation for Use:** Reconstitute the vial with **1 mL** distilled water. Mix well, do not shake and leave at room temperature for 10 minutes before use.

**Storage and Stability:** The lyophilized reagent is stable until the expiration date printed on the vial. After reconstitution, the reagent is stable for 48 hours at 2-8° C. The reagent may be stored at -20° C for 1 month. Thaw rapidly at 37° C before use.

**WARNING: SODIUM AZIDE.** Both **LupoTek DetecTin** and **LupoTek CorrecTin** contain sodium azide, which can form highly explosive metal azides if exposed to lead or copper in plumbing. Any such materials should be discarded into a sink only with large volumes of water to minimize such a risk.

### TECHNIQUES

The DRVVT test may be performed either by acceptable manual methods or using optical or electromechanical coagulation analyzers. Adaptation protocols for most analyzers are available on request from R<sup>2</sup> Diagnostics.

### SPECIMEN COLLECTION AND PREPARATION

**Specimen:** Plasma obtained from whole blood anticoagulated with 0.1M sodium citrate.

**Specimen Collection:** Nine parts freshly collected whole blood should be immediately added to one part anticoagulant and mixed thoroughly.

**Specimen Preparation:** Centrifuge the whole blood at 2500 x g for 15 minutes (NCCLS H21-A2,1991). Immediately separate the plasma from the red cells using a plastic pipette and place in a plastic test tube. To insure an optimum sample, the plasma should contain less than 10 x 10<sup>9</sup>/L platelets. (6). For platelet free plasma, filtration through a 0.22 micron (μ) syringe type filter before testing is recommended (7). This is particularly important if plasma is to be frozen before testing.

**Storage and Stability:** Before and during testing, the plasma should be stored at 4° C and is stable for up to 4 hours. Alternatively, samples may be frozen at -20° C or below for up to 1 month. It is strongly recommended that plasma should be filtered before freezing as outlined above. Any residual platelets will rupture on freezing and thawing and can neutralize a lupus anticoagulant by exposure of phospholipids from the damaged membranes. Thaw rapidly at 37° C before use.

### TEST PROCEDURE

#### Materials Provided:

**LupoTek DetecTin**

100 determinations

10 vials DRVVT test for detecting Lupus Anticoagulants, 2 mL.

**LupoTek CorrecTin**

100 determinations

10 vials Phospholipid Rich Lupus Correcting Reagent, 1 mL.

#### Materials required but not provided:

1. Plastic test tubes- 12 x 75 mm
2. 200 μL precision pipette
3. Stopwatch or appropriate timing device
4. 37° C water bath or coagulation analyzer
5. Distilled water
6. Platelet poor normal pool plasma

### STEP-BY-STEP METHOD

#### A. Specimen and Reagent Preparation

1. All test tubes, pipette tips and syringes should be plastic
2. Collect and prepare the blood sample specimen according to the directions outlined in the **SPECIMEN COLLECTION AND PREPARATION** section
3. Prepare the reagents according to the reconstitution instructions in the **REAGENTS** section.

#### B. Testing of Samples

1. Place an appropriate amount of **LupoTek DetecTin** and **CorrecTin** in the waterbath or reagent reservoir of an instrument. Allow 200μl per test and allow to warm to 37° C before use.
2. Add 200μL of normal control or test plasma to a plastic test tube and incubate at 37° C for 1 minute.
3. Add 200μL of the prewarmed **LupoTek DetecTin** or **CorrecTin** to the test plasma, simultaneously starting a stopwatch and determine the time taken for the sample to clot.
4. Repeat to obtain duplicate results, and average the two clotting times.

#### Quality Control

Quality control of coagulation tests involves multiple components. Each laboratory should establish a quality control program that includes both normal and abnormal control plasmas. All control plasmas should be tested and validated before performing any testing on patient plasmas.

If the control plasmas are outside their reference ranges, no patient results should be reported.

Quality control testing with the **LupoTek DetecTin** and **CorrecTin** reagents should be carried out at the same time.

**RESULTS**

For optimum results, testing using **LupoTek DetecTin** and **CorrecTin** should be done at the same time. If the **LupoTek DetecTin** time is in the normal range no further testing is necessary.

If the clotting time with the **DetecTin** reagent is longer than **20%** above the mean of the normal reference range, then further testing with the **CorrecTin** reagent is justified.

The clotting times obtained with the **DetecTin** and **CorrecTin** reagents are used to express results in a ratio format, as outlined below. The use of a **normalized ratio** in which the patient’s clotting time is divided by the clotting time of the normal control, minimizes any impact of differences in the normal ranges due to lot to lot reagent variability

$$\frac{\text{DetecTin Time - Patient}}{\text{DetecTin Time - Normal Control}}$$

**RATIO**

$$\frac{\text{CorrecTin Time - Patient}}{\text{CorrecTin Time - Normal Control}}$$

**INTERPRETATION OF RESULTS**

If the RATIO is less than 1.2 - No LA is present

If the RATIO is between 1.2 and 1.5 - A weak LA is present

If the RATIO is between 1.5 and 2.0 - A moderate LA is present

If the RATIO is greater than 2.0 - A strong LA is present

The **LupoTek CorrecTin** reagent has a high lipid concentration making it less sensitive to the presence of LA’s in the patients’ plasma. Any normal patient or normal control plasma should give similar results with both the **LupoTek DetecTin** and **CorrecTin** reagents. It must be remembered that the cutoff values assigned to these ratios are not absolute and each laboratory should establish its own specific ratios for its normal reference and patient populations. Patient plasmas yielding borderline ratios ( 1.2 - 1.3) should be repeated and correlated with the clinical findings if indicated. Patient plasmas that have long clotting times with both **LupoTek DetecTin** and **CorrecTin** irrespective of the ratio, may have some other defect such as a Factor deficiency state or be on oral anticoagulant therapy.

**Mixing Studies**

The differentiation between factor deficiency states and circulating inhibitors can be optimized by doing mixing studies with normal plasma. A failure to correct on mixing with normal plasma (1:1) is more indicative of an inhibitor, while correction is more suggestive of a factor deficiency state. Mixing studies should be carried out under carefully controlled conditions using a well characterized pooled normal plasma with stringent attention to incubation times etc.

Using a combination of mixing tests with **LupoTek DetecTin** and **LupoTek CorrecTin** testing may help in identifying the cause of abnormal test results. An example using combined test data is given in Table 1.

**TABLE 1**

LupoTek DetecTin		LupoTek CorrecTin		Diagnosis
Patient	Mix 1:1	Patient	Mix 1:1	
N	N	N	N	Normal
ABN	ABN	N	N	LA Present
ABN	N	ABN	N	Factor Deficiency
ABN	ABN	ABN	N	LA + Factor Deficiency
ABN	ABN	ABN	ABN	Other Inhibitor

**REFERENCE VALUES**

Reference ranges for normal plasmas with **LupoTek DetecTin** are in the **28-42** second range.

Reference ranges for normal plasmas with **LupoTek CorrecTin** are in the **25-35** second range.

The **reference ratio** of DetecTin:CorrecTin for normal plasmas was **0.8-1.2**. These results are to be considered a guide only and each laboratory must establish its own normal references ranges for the technique and/or instrument used in the laboratory.

**LIMITATIONS**

All testing for lupus anticoagulants require plasma samples that are platelet poor (<10 x 10<sup>9</sup> /L) or preferably platelet free. Samples should be tested before freezing since any platelets left will rupture and expose phospholipids. This exposure can neutralize the LA especially a weak one and cause a false normal result. Caution must also be exercised if the **LupoTek DetecTin** is performed on fresh sample whereas the **LupoTek CorrecTin** is subsequently performed on a frozen sample.

**PERFORMANCE CHARACTERISTICS**

**1. Precision**

Precision studies were performed to establish within run and between run CV’s for normal and abnormal samples. Values of 3.5% were obtained on normal plasmas and 5% on abnormal plasmas.

**2. Specificity**

Studies were done on known patient plasma samples. Using the positive cut-off **ratio** as > 1.2, and expressing the results as a percentage, the results were as follows:

LA plasmas	90%
Heparinised plasmas	12%
Oral anticoagulant plasmas	0%
Factor deficiency plasmas	0%
Normal plasmas	2%

**References:**

1. Love PE. et al. Antiphospholipid antibodies: Anticardiolipin antibodies and the lupus anticoagulant in SLE. and non-SLE disorders. Ann. Intern. Med: 1990; 112, 682-698.
2. Ginsberg JS et al. Relationship of antiphospholipid antibodies to pregnancy loss in patients with SLE: A cross sectional study Blood:1992; 80:4
3. Ames PRJ. et al. Antiphospholipid antibodies, hemostatic variables and thrombosis-A survey of 144 patients. Thromb. Haemost: 1995; 73: 768-773.
4. Triplett DA. et al. Laboratory identification of the lupus anticoagulant. Br. J. Haematol: 1991; 73; 139-142.
5. Exner T. et al. Use of a simplified dilute Russell’s viper venom time (DRVVT) confirms heterogeneity among “ lupus anticoagulants “. Blood Coag. Fibrinol: 1990; 1, 259-266.
6. Brandt JT. et al. Criteria for the diagnosis of Lupus Anticoagulants: An update. Thromb. Haemost: 1995;74(4) 1185-1190.
7. Sletnes KE. et al. Preparation of plasmas for the detection of lupus anticoagulants and antiphospholipid antibodies. Thromb. Res: 1992; 65. 43-53

This example should not be used to draw conclusions about any patient sample but is simply a reference point to consider in evaluating abnormal test findings.