MediRox

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Changes: New logotype and text format. Adjusted text regarding precautions referring to MSDS
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MRX APTT Liquid Reagent Art. No: MRX930/MRX931

INTENDED USE

For the in vitro determination of the Activated Partial Thromboplastin Time (APTT) as a procedure for the global evaluation of the intrinsic pathway ([1]) and for monitoring heparin anticoagulant therapy ([2-4]) as well as for use in other coagulation methods where an APTT reagent is required.

FOR IN VITRO DIAGNOSTIC USE

BACKGROUND AND PRINCIPLE OF METHOD

In the APTT test a contact activator is used to stimulate the generation of Factor XIa by providing a surface for the interaction of FXI, high molecular weight kininogen, kallikrein and Factor XIIa ([1]).

The contact activation is allowed to proceed at 37 °C for a specific period of time. Calcium chloride is then added to trigger further reactions and the time required for formation of a fibrin clot is determined. Phospholipids are required to form complexes which activate Factor X and prothrombin.

The APTT reagent in the kit contains phospholipids and silica to ensure a highly consistent and stable product ([5]).

The APTT reagent is lupus anticoagulant insensitive ([6-7]). Lupus anticoagulant insensitive reagents yield more reliable factor assay results than reagents which are sensitive to lupus inhibitors. Lupus inhibitors occur occasionally among test plasmas.

Prolonged clotting times may be observed in the following situations: deficiency of Factor XII, XI, X, IX; VIII; V, II or fibrinogen, in liver diseases, vitamin K deficiency, presence of heparin, lupus anticoagulant or other inhibitors, which affect the intrinsic pathway ([8]). A shortened APTT appears to correlate with an increased risk of venous thrombosis ([9]).

PRODUCT DESCRIPTION

MRX930 contains:

- APTT reagent: 5 X 5 mL vials of colloidal silicate with phospholipids, buffer and preservatives.
- Calcium Chloride: 5 X 5 mL vials of calcium chloride 0,025 mol/L with preservatives.

MRX931 contains:

- APTT reagent: 5 X 10 mL vials of colloidal silicate with phospholipids, buffer and preservatives.
- Calcium Chloride: 5 X 10 mL vials of calcium chloride 0,025 mol/L with preservatives.

PRECAUTIONS

The product contains Sodium Azid (<0,1%) to prevent bacterial growth. Do not empty into drains.Avoid contact with skin and eyes.

For more information refer to Material Safety Data Sheet

PREPARATION

- APTT reagent: Mix before use.
- Calcium Chloride: The reagent is ready for use.

STORAGE CONDITIONS AND STABILITY

Unopened reagents are stable until the expiration date shown on the label when stored at 2-8°C.

APTT reagent: Opened reagent is stable for 30 days at 2-8°C in the original vial.

Calcium Chloride: Opened reagent is stable for 30 days at 2-25°C in the original vial.

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection and storage be carried out in accordance with the CLSI guideline H21-A5 ([10]).

Venous blood is collected in 3.2% or 3.8% sodium citrate at a ratio of 9 parts blood to 1 part anticoagulant (1:10 ratio). The ratio is critical. If using commercial vacuum tubes, a full draw must be assured. Trauma or stasis during blood sampling should be avoided. Blood should not be collected through a heparin lock or other heparinized line. The presence of a clot in a specimen is a cause for rejection.

Centrifuge at 1500 x g for 15 minutes or at a speed and time required to produce platelet poor plasma (platelet count < 10,000/µl). Unless samples are to be processed immediately, transfer plasma to a plastic tube as soon as centrifugation is completed. Plasma samples can be stored at room temperature (18-26°C) for up to 4 hours; refrigerated (2-8°C) for up to 4 hours; frozen at -20°C for up to 2 weeks or at -70°C for up to 6 months. Frozen samples should be thawed rapidly, mixed and tested immediately or within 2 h after storage at 2-8°C. No contact with glass should occur.

PROCEDURE

This procedure pertains to a manual or semi-automated coagulation system. Refer to your instrument manual for more detailed instrument specific instructions.

1. Preincubate Calcium Chloride at 37°C for at least 10 minutes
2. Pipette 100 ul of sample into a test cuvette. Incubate at 37°C for 1-2 minutes.
3. Add 100 ul APTT reagent to the cuvette containing the plasma. Incubate the mixture at 37°C for 5 minutes.
4. Add 100 ul of Calcium Chloride (37°C) and simultaneously start the timer.
5. Record the clotting time in seconds.

QUALITY CONTROL

MediRox recommends the use of Normal control plasma (GHI 162, GHI163, GHI164) and Abnormal control plasma (GHI 167B, GHI169, GHI170) for reliable quality control of the performance and at a frequency in accordance with good laboratory practise.

LIMITATIONS AND INTERFERENCES

Delay in testing and difficulty in specimen collection may result in falsely prolonged APTT results. Hence, a strict control of plasma preparation and handling is of importance.

Inadvertent preactivation of coagulation factors may result in shortening of the APTT.

The impact of coagulation factor deficiency on the APTT may be compensated for by preactivation and by elevated levels of other coagulation factors.

APTT results may be affected by certain drugs.

APTT results are not affected by hemoglobin up to 10 mg/mL and bilirubin up to 0,50 mg/mL.

EXPECTED VALUES

APTT results are influenced by the method of clot detection and can vary from laboratory to laboratory.

Each laboratory is recommended to establish its own normal range on the specific instrument used.

TYPICAL PERFORMANCE (ACL 9000)

	CV% (within run)	CV% (between runs)
Normal control	1,0	1,5
Abnormal control	1,0	1,5

Factor Sensitivity:

	APTT-Clotting Time (in seconds) Normal range 26,7-34,9 sec.		
Factor (%)	F VIII	F IX	FXI
<1	85,7	70,9	92,4
10	45,9	44,4	50,7
40	34,2	33,9	34,4
100	28,8	28,8	28,8

Factor sensitivity testing was carried out by mixing varying volumes of pooled normal plasma (PNP, 100% factor levels) with individual intrinsic factor deficient plasma to achieve 10% and 40% factor levels. Results with PNP and factor deficient plasmas alone are also shown in the table above. All tests were run with 5 minutes preincubation on ACL300R. Normal range shown is mean +/- 2 SD.

These values should be used as guidelines only. Each laboratory should establish factor sensitivity using their own instruments and techniques.

Heparin Sensitivity:

Heparin U/mL	APTT clotting time in seconds
0	29,9
0,2	70,0
0,4	174

Results were obtained using PNP spiked with unfractionated heparin. All tests were run with 5 minutes preincubation on ACL300R.

Due to many variables (e.g. different sources of heparin) wich may affect the clotting times, each laboratory should establish its own heparin therapeutic range.

REFERENCES

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