Diagnostic Reagents Limited

UK CA

Micronised Silica Platelet Substitute Mixture

Catalogue Number: MSPS060 (5 mL)

For *in vitro* diagnostic use only.

Intended Use
Diagen Micronised Silica Platelet Substitute Mixture is designed for use in the *in vitro* determination of the Activated Partial Thromboplastin Time (APTT).

Summary and Principle
The Activated partial thromboplastin time (APTT) has developed from the partial thromboplastin time (PTT), the re-calcification time and the whole blood clotting time. It is a measure of the combined effect of the clotting factors of the Intrinsic and common coagulation pathways. It represents the ultimate refinement in which platelet activity is standardised by the use of platelet substitute and contact activation is standardised by pre-incubation of the plasma with the Micronised Silica Platelet Substitute Mixture for a standard time before re-calcification[1]. At 5 minutes pre-incubation, the normal range for this test is 28-43 seconds (40 -150% factor VIIIc). Plasma samples completely deficient in factors VIII or IX give clotting times in the region of 120 seconds, and minor deficiencies of these factors should result in an APTT prolonged beyond the normal range.

Because of the 'broad spectrum' nature of this test, it will reflect deficiencies of any of the factors of the intrinsic or common clotting pathways (Factors II, V, VIII, IX, X, XI, XII & Fibrinogen) and is therefore a valuable ancillary screening test. The reagent is also **highly** sensitive to therapeutic levels of Heparin & the Lupus antigoagulant.

Reagent
Micronised Silica Platelet Substitute Mixture 6 vials
A lyophilised colloidal dispersion of micronised silica, with added bovine phospholipid, buffers and preservatives. For reconstitution, remove cap and rubber bung, add the required volume of distilled water (5.0 mL) to the contents of the vial and allow 5 – 10 minutes for complete solution. The vial now contains a suspension of micronised silica in platelet substitute.

Warnings and Precautions
Diagen Micronised Silica Platelet Substitute Mixture contains components sourced from animal origin, passed fit for human consumption. Reagents containing animal products should be treated as potentially infectious. All wastes containing biological material should be correctly labelled and stored separately from other wastes. Waste materials should be disposed of according to prescribed international, national and local regulations. Please refer to the SDS Sheet (provided on request) for handling and safety procedures.

Collection of Blood Samples
Blood (9 parts) is collected into 1 part of 3.2% trisodium citrate and the plasma obtained by centrifugation at 2500 g for 15 minutes. The plasma should be stored in stoppered tubes. The use of 3.2% citrate containing 5% HEPES buffer improves the stability of both fresh and deep frozen plasma.

Procedure
Materials Provided
Material needed for Activated Partial Thromboplastin Time tests (APTT) are:
Cat. No.
MSPS060 – Micronised Silica Platelet Substitute Mixture (6 x 5 mL vials).

Materials and equipment required, but not provided:
1. General routine laboratory coagulation equipment.

2. Reaction cups or test tubes (12 x 75 mm).
3. Pipettes delivering: 100 µL, 200 µL & 5.0 mL.
4. Distilled water.
5. 25mM $CaCl_2$ solution (CTMM542).
6. Diagen Control plasmas: IQCN130 - Normal.
   IQCM140 - Abnormal 1 (Mild).
   IQCS150 - Abnormal 2 (Severe).

Manual Technique
1) 100 µL of Micronised Silica/Platelet Substitute Mixture is placed in a clotting tube in a 37°C water bath and incubated for 1–2 minutes to reach temperature.
2) 100 µL of test plasma (or control) is added and the tube gently tilted at intervals for **exactly five minutes.**
3) 100 µL of 0.025 M calcium chloride (pre-incubated at 37°C) is then added and a stopwatch started.
4) The tube is tilted at regular intervals and the time for clot formation is recorded.
5) The test is carried out in duplicate for both the control and the patient's sample, and the mean value for each obtained. This clotting time is called the Activated Partial Thromboplastin time (APTT).

Notes:
1) Tubes should be new and scrupulously clean.
2) Water bath temperature should be 37°C.
3) Our freeze-dried normal plasma can be used as a normal control and day-to-day QC.
4) For photo-optical and mechanical instruments, follow the manufacturer's instructions.

Quality Control
All laboratories should have in place a quality control system that uses normal and abnormal controls to evaluate reagent, instrument and user performance. Both normal and abnormal controls should be used prior to performing a test series to validate the patient results. We recommend Diagen control plasmas for this purpose, as these have been specifically manufactured for our reagents. If the controls do not perform within their reference ranges, a review of the instrument or test system is recommended.

Interpretation
Deficiency of factors II, V, VIII, IX, X, XI, XII & Fibrinogen should result in a prolonged clotting time. See also the Heparin and Lupus anticoagulant section below.

Reporting Results
The test APTT is recorded in seconds and compared to a pre-determined reference range. Alternatively, the patients clotting time can be divided by the mean normal clotting time and recorded as a ratio.

Normal Range
The normal range should be determined locally in each laboratory, especially where photo optical or mechanical instruments are used. This may be obtained cumulatively by testing individual fresh normal plasma samples at the same time keeping the method "in control" by the use of a freeze-dried plasma control as a test of reagent, water bath temperature, calcium chloride etc. The normal range quoted is that obtained using a photo optical instrument.

Performance and Sensitivity [2]
Precision of replicate clotting times
20 replicate determinations on each of a normal and abnormal sample gave the following % CV's.

	No.	Manual	Photo-optical
Normal	20	1.2	1.1
Abnormal	20	1.6	1.6
4 hours later			
Normal	20	1.6	1.4
Abnormal	20	1.9	1.8

Between-day precision
A normal lyophilised plasma was tested daily for 20 days and gave the following % CV's.

Manual	Photo Optical
1.8	2.0

Sensitivity to Heparin
In our hands, patients receiving therapeutic levels of unfractionated heparin (0.2 to 0.5 u/mL) give the clotting time ratios of:

$$\frac{\text{Patient plasma clotting time}}{\text{Normal plasma clotting time}} = 2.0 \text{ to } 3.5$$

Sensitivity to Lupus inhibitors
For prolonged clotting times the following method is useful:
1. Reconstitute 1 vial of Micronised silica Platelet Substitute Mixture in half the recommended volume in distilled water (x2 strength).
2. Reconstitute a second vial at normal strength in distilled water (x1 strength).

The following patterns of clotting times are obtained:

	Vial 1 (x2 strength)	Vial 2 (x1 strength)
Normal Plasma (sec)	35	35
Factor deficiency (sec)	76	69
Ratio Abn/Norm	2.17	1.97
Lupus Inhibitor (sec)	100	136
Ratio Abn/Norm	2.85	3.89

Clotting factor deficiencies give little change or slight increase in ratio at x 2 strength, and Lupus Inhibitors give a marked decrease in ratio at x 2 strength.

Limitations
APTT values will differ between laboratories due to the many variables that can affect clotting times in particular, the use of coagulometers. All laboratories should therefore establish a quality control system that uses well-defined performance standards for control plasmas. The use of icteric, lipemic, or haemolyzed samples should be avoided as this may cause possible interference, especially when using photo-optical instruments. If the patient is on therapeutic drugs, it may influence interpretation of APTT test results. By obtaining accurate patient history and noting specific drug therapies we can better understand the potential impact on laboratory test results. The presence of heparin as a contaminant must always be considered in a sample where an abnormal result is obtained.

It should be remembered that a normal result obtained with this test might not exclude borderline or minor Factor deficiencies.

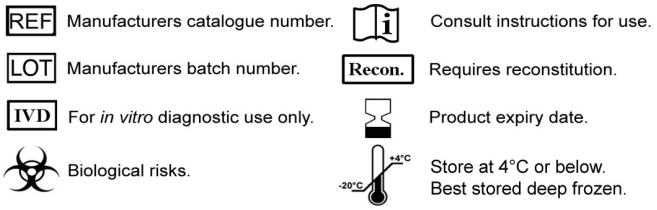
Storage and stability
Best stored Deep frozen. The freeze-dried material in the unopened vial can be stored at 4°C or below for 3 years after manufacture without any deterioration. After reconstitution, the suspension is stable for at least 2 weeks at 4°C and should not be frozen.

Packaging
6 x 5 mL.

References
1. Denson, K.W.E. (1976) IN "Human Blood Coagulation, Haemostasis and Thrombosis'. (Ed. R. Biggs). Blackwell Scientific Publications, Oxford, London, Edinburgh and Melbourne.
2. Koepke. J.A. (1986) ICSH Panel on the PTT. Thrombosis and Haemostasis 55 (1) 143-144

MSPSIFU-006 Revision -

Key guide to symbols



Manufacturer.

Diagnostic Reagents Ltd.
Thame
Oxon, OX9 3NY
UK
Tel:+44(0)1844 212426
Email: sales@diagen.co.uk
Website: www.diagen.co.uk

Diagnostic Reagents Limited is a BS EN ISO13485:2016 certified company

© Diagnostic Reagents Ltd 2022