Diagnostic Reagents Limited

UK CA

Kaolin Platelet Substitute Mixture

Catalogue Number: KAPS050 (5 mL)

For *in vitro* diagnostic use only.

Intended Use
Diagen Kaolin Platelet Substitute Mixture is suitable for use in the Activated Partial Thromboplastin time (APTT) or Kaolin Cephalin clotting time (KCCT).

Summary and Principle
The Activated partial thromboplastin time (APTT) or Kaolin cephalin clotting time (KCCT) has developed from the partial thromboplastin time (PTT), the recalcification 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 Kaolin Platelet Substitute Mixture for a standard time before re-calcification [(1)]. At 2 minutes pre-incubation, for the manual method, the normal range for this test is 36-50 seconds (40-150% factor VIIIc) and at 5 minutes preincubation, 32-45 seconds. 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 a clotting time 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 sensitive to therapeutic levels of Heparin & the Lupus anticoagulant.

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.

Reagent
Kaolin Platelet Substitute Mixture 6 vials
A lyophilised mixture of Kaolin and Phospholipid. For reconstitution, remove cap and rubber bung, and then add the required volume of distilled water (5.0 mL) to the contents of the vial. The vial now contains a suspension of kaolin in platelet substitute. The kaolin tends to settle on standing and **blowing one or two small air bubbles through the suspension before removing a volume should agitate the contents of the vial.**

Warnings and Precautions
Diagen Kaolin Platelet Substitute Mixture contains components sourced from animal origin, passed fit for human consumption. However, reagents containing animal products should be treated as potentially infectious. All wastes containing biological material should be correctly labeled 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.

Procedure
Materials Provided
Material needed for the Activated Partial Thromboplastin time tests (APTT) or KCCT are shown on page two:

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Cat. No.
KAPS050 - Kaolin 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. Control plasmas: IQCN130 - Normal.
   IQCM140 – Abnormal 1 (Mild).
   IQCS150 – Abnormal 2 (Severe).

Manual Technique
1) 200 µL of Kaolin 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 two 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) or Kaolin Cephalin Clotting Time (KCCT)

Notes:
1) Tubes should be new and scrupulously clean.
2) Water bath temperature should be 37°C.
3) For photo-optical and mechanical instruments, follow the manufacturer's instructions.

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 the manual method.

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 or KCCT 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.

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

	No.	Manual	Photo-optical
Normal	20	1.2	1.1
Abnormal	20	1.6	1.7
4 hours later			
Normal	20	1.6	1.5
Abnormal	20	1.9	2.0

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

Manual	Photo Optical
1.8	2.1

Sensitivity to Factor VIII
The following table shows the clotting times in seconds and the plasma factor VIIIc concentration (% of normal). The activation time used was 2 minutes.

VIII Conc. %	150	100	80	60	50	40	20	10	5	1
CT (Secs)	36.0	40.0	42.0	45.5	48.0	50.0	58.0	65.5	73.0	91.5

The log factor VIIIc % plotted against the clotting time in seconds gives a straight line the slope of which can be expressed as follows:

$y = m (\log x) + K$

Where……
y = APTT in seconds
m = slope = -2
x = % Factor VIIIc
k = constant = 91.5

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:

$$\frac{\text{Patient plasma clotting time}}{\text{Normal plasma clotting time}} = 1.4 \text{ to } 2.5$$

Sensitivity to Lupus or APL inhibitors
Diagen Kaolin Platelet Substitute Mixture reagent is moderately sensitive to the lupus inhibitor. However, as a screening test, using a 1 in 10 dilution of reagent in Imidazole buffer can increase sensitivity. Alternatively, Diagen Micronized Silica Platelet Substitute Mixture can be used in parallel, which has a very high sensitivity to Lupus or APL antibodies

Limitations
APTT values will differ between laboratories due to the many variables that can affect clotting times, particularly 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 two years from the date of 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.

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Key guide to symbols

REF	Manufacturers catalogue number.		Consult instructions for use.
LOT	Manufacturers batch number.	Recon.	Requires reconstitution.
IVD	For *in vitro* diagnostic use only.		Product expiry date.
	Biological risks.	+4°C -20°C	Store at 4°C or below. Best stored deep frozen.

Manufacturer.

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

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