SIEMENS Healthineers

Dade® Actin® FS Activated PTT Reagent

ACTIN FS

Revision bar indicates update to previous version.

Intended Use

Liquid purified soy phosphatides with plasma activator for use in the determination of the activated partial thromboplastin time (APTT) and other coagulation procedures requiring an activated partial thromboplastin reagent.

Summary and Explanation

The activated partial thromboplastin time, a global screening procedure[1,2] used primarily to evaluate coagulation abnormalities in the intrinsic pathway, will also detect severe functional deficiencies in factors II, V, X, or fibrinogen. The APTT has also been widely advocated[3–6] as a means to monitor the effectiveness of unfractionated heparin therapy where the clotting time is prolonged in proportion to the level of heparin. In patients receiving oral anticoagulants, the circulating levels of factors II, VII, IX, and X are depressed, therefore the APTT can be expected to be prolonged. The presence of non-specific inhibitors, such as the lupus-like anticoagulant[1,8], may prolong the APTT but this effect is variable and generally recognized as being related more to the nature of the APTT reagent employed. In summary, the APTT is a clinically important screening test with wide applicability for the diagnosis of coagulant disorders and therapeutic monitoring of both hemorrhagic and thrombotic disease.

Principles of the Procedure

Factors of the intrinsic coagulation system are activated by incubating the plasma with the optimal amount of phospholipids and a surface activator. The addition of calcium ions triggers the coagulation process, and the clotting time is then measured.

Reagents

Note: ACTIN FS can be used on automated coagulation analyzers. Siemens Healthineers provides Reference Guides (Application Sheets) for several coagulation analyzers. The Reference Guides (Application Sheets) contain analyzer/assay specific handling and performance information which may differ from that provided in these Instructions for Use. In this case, the information contained in the Reference Guides (Application Sheets) supersedes the information in these Instructions for Use. Please also consult the instruction manual of the instrument manufacturer!

Reagent	Description	Storage	Stability
Dade® Actin® FS Activated PTT Reagent ACTIN FS	Ready to use liquid containing: • purified soy phosphatides[a] in 1.0×10^{-4} M ellagic acid • Buffer • Stabilizer • Preservative	2–8 °C May be used up to the expiry date indicated on the label if stored unopened. Do not freeze!	2–15 °C: once opened, 7 days[b]

[a] No standard potency has been established and accepted for purified soy phosphatides.

[b] closed original vial

If the reagent is left to stand, a green deposit may form consisting of ellagic acid and lipids. Before use, mix by inverting. Avoid contamination with plasma.

Signs of expiry: Deviations from the normal laboratory value in the determination of normal plasma or controls.

On-board stability
Information regarding on-board stability is specified in the Reference Guides (Application Sheets) for the different coagulation analyzers.

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

Safety data sheets (MSDS/SDS) available on siemens-healthineers.com/sds.

CAUTION!
Federal (USA) law restricts this device to sale by or on the order of licensed healthcare professionals.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Preparing Reagents

ACTIN FS must be mixed gently by inversion (5 to 8 times) before use.

Specimen Collection and Handling

Collecting the Specimen

Mix nine parts of freshly collected patient blood with one part of 0.11 mol/L or 0.13 mol/L (3.2 % or 3.8 %) sodium citrate[22,23]. It is recommended that blood specimens for plasma-based coagulation testing should be collected by venipuncture using a blood collection system that collects the specimen directly into a glass or plastic evacuated tube containing the appropriate additive.

Evacuated tubes containing the desired anticoagulant are commercially available and may be used with caution in blood coagulation studies.

For special studies, syringe technique may be preferred.

Centrifuge the blood specimen at 1 500 × g for > 15 minutes at room temperature as soon as possible after collection.

Storing the Specimen

Store in an unopened tube at room temperature.

If immediate testing is to be done, the plasma may remain on the packed cells. Otherwise plasma should be separated from the cells. To separate the plasma, use a plastic transfer pipette, remove plasma to a plastic tube.

Do not store on ice.

Non-heparinized plasma should be tested within 4 hours of blood collection.

Plasma containing unfractionated heparin should be centrifuged within one hour of blood collection, stored at room temperature and tested within 4 hours[24].

Platelet-poor plasma may be frozen at ≤ −20 °C for up to 2 weeks in a non frost-free freezer. Frozen plasma should be rapidly thawed at 37 °C, gently mixed and tested immediately. Samples should not stand at 37 °C for > 5 minutes[25].

Please refer to CLSI document H21–A5[9] for detailed information on sample preparation and storage.

Procedure

Materials Provided

REF	Contents		
B4218-20	Dade® Actin® FS Activated PTT Reagent ACTIN FS	10 ×	2 mL
B4218-100	Dade® Actin® FS Activated PTT Reagent ACTIN FS	10 ×	10 mL

Materials Required but not Provided

Item	Description
REF ORHO37	$CaCl_2$ SOLUTION, Calcium Chloride Solution, (0.025 mol/L)
REF ORKE45 REF B4244-10	CONTROL N, Control Plasma N, or Ci-Trol CONTROL 1, Dade® Ci-Trol® Coagulation Control Level 1, as control for the normal range
REF OUPZ19 REF B4244-20	CONTROL P, Control Plasma P, or Ci-Trol CONTROL 2, Dade® Ci-Trol® Coagulation Control Level 2, as control for the pathological/therapeutical range
REF B4244-30	Ci-Trol CONTROL 3, Dade® Ci-Trol® Coagulation Control Level 3, as control for the pathological/therapeutical range
REF B4224-50	Ci-Trol HEPARIN CONTROL 1, Dade® Ci-Trol® Heparin Control, Low
REF B4224-60	Ci-Trol HEPARIN CONTROL 2, Dade® Ci-Trol® Heparin Control, High
–	For blood collection, use sodium citrate (0.11 mol/L or 0.13 mol/L / 3.2 % or 3.8 %), or Standard commercial blood collection systems
–	Distilled or deionized water without preservatives
–	Plastic test tubes
–	Pipettes for precise measurement of 0.1 mL
Coagulation analyzers, such as:	• BCS® XP System • BFT *II* Analyzer • SYSMEX CA-600 series • SYSMEX CA-1500 System • SYSMEX CS-2500 System • SYSMEX CS-5100 System

Manual Testing

Pre-warm $CaCl_2$ SOLUTION at 37 °C		
Pre-warm 0.1 mL ACTIN FS for 1 minute at 37 °C. (Mix before use)		
Pipet into coagulation tubes as follows:		
	Test Sample	Control Plasma
ACTIN FS (pre-warmed)	0.1 mL	0.1 mL
Plasma	0.1 mL	–
Control Plasma	–	0.1 mL

	Mix well. Incubate at 37 °C for 3 minutes.	
$CaCl_2$ SOLUTION (pre-warmed)	0.1 mL	0.1 mL
	Simultaneously with addition of $CaCl_2$ SOLUTION start stopwatch, mix well. After 20 seconds start to observe for clot formation.	

Note: Incubation times exceeding 5 minutes may cause loss of FV and FVIII and are not recommended. Each laboratory should determine the optimal heating-activation time for its particular assay system.

Monitoring of Unfractionated Heparin Therapy with APTT

When using the APTT for this purpose, the factors influencing the test should be kept in mind. General considerations are listed below.

A. Time of collection is important since the *in-vivo* half-life of unfractionated heparin is approximately 1.5 hours[5]. When it is administered, it has an immediate anticoagulant effect but the degree of this effect decreases rapidly with time. This is especially apparent with intermittent single intravenous injections.

B. The anticoagulant used for sample collection can alter test results.

C. Platelet factor 4, a heparin neutralizing factor in platelet alpha-granules, can be released by platelet aggregation or damage. To prevent this occurrence *in-vitro*, the specimen should be collected with a minimum of trauma. Cold temperatures are known to induce platelet aggregation and release platelet factor 4; therefore, centrifugation at room temperature is recommended for heparin studies.

D. Using APTT to monitor unfractionated heparin therapy is time-dependent. Delay in testing samples will result in prolonged APTT determinations. Therefore, it is imperative that the testing on all samples be performed as soon as possible.

E. Increased contact activation times may result in prolonged APTT in plasma containing heparin. It is imperative that the optimal heating-activation time of the ACTIN FS-plasma mixture be rigidly standardized[10].

F. Different test systems (i.e., manual, photo-optical, etc.) will exhibit variable heparin sensitivity. Interchanging of test systems should be avoided.

G. Baseline data on the APTT of each patient before the start of therapy should be established where feasible to determine the respective patient APTT as it relates to the normal range established for the test in that laboratory.

H. Studies have shown variability in original estimates of the quality of unfractionated heparin from different sources and different manufacturers. *In-vivo* reactivity varies with the type of heparin administered, the metabolism of the individual and other co-administrated medications[5,7].

I. Because the APTT can vary with technique, method, equipment, reagent lot and heparin used, each laboratory must establish its own therapeutic ranges, or verify them whenever one or more of the aforementioned variables is changed. This can be done by simultaneously determining the APTT and the heparin concentration for samples from patients receiving heparin therapy. A dose-response curve can be calculated from the data using regression analysis, and the APTT range corresponding to a heparin concentration of 0.3 to 0.7 U/mL (by a factor Xa inhibition assay) can be derived[4,5,7].

Internal Quality Control

Normal range:	Ci-Trol CONTROL 1, or CONTROL N
Pathological range:	Ci-Trol CONTROL 2, or Ci-Trol CONTROL 3, or CONTROL P
Heparin monitoring:	Ci-Trol HEPARIN CONTROL 1 Ci-Trol HEPARIN CONTROL 2

Two controls (one in the normal range and one in the pathological/therapeutical range) must be measured at the start of the test run, after each change of reagent vial, and at least once during an 8-hour shift. The control material should be prepared and processed in the same manner as the patient samples. Each laboratory should establish its own confidence intervals for the controls. This interval is generally ±2 to ±2.5 standard deviations (SD) from the mean control value. If the control values are

outside of the confidence interval, the controls, reagents and instrument must be checked. Before reporting the patient values, it is recommended that all steps should be documented that were taken to identify and rectify the problem. New control ranges should be defined for each new lot of reagents or controls.

Results

Results of the activated partial thromboplastin time testing should be reported as the APTT in seconds. These results should be related to the normal range for APTT testing in each laboratory. It is suggested that the patient results be reported to the clinician in conjunction with the normal range. Control values for the reagent test system should never be used in place of a normal range. Furthermore, the reporting of APTT results in terms of an upper normal only may result in incorrect interpretation. Shortened APTT results may also indicate some abnormal condition in the patient's coagulation system.

Limitations

APTT testing encompasses the entire clotting process from contact activation to fibrin formation and is therefore more susceptible to variations than specific individual tests. The control and use of APTT is therefore subject to inherent limitations. Control of plasma sample conditions is strictly emphasized. Studies have shown that sample decomposition may occur more rapidly in stored samples that are not refrigerated. Extremely small plasma volumes (prior to testing) are to be avoided since pH changes in the plasma from physiological conditions may be encountered. Such changes may lead to the decomposition of plasma components of the blood coagulation system.

It should be noted that APTT testing may be affected by a number of commonly administered drugs. Decrease in time of APTT determination in conjugated estrogen therapy in males and oral contraceptive administration in females has been reported[11,12]. Increase in the APTT has been seen in diphenylhydantoin, heparin, warfarin, naloxone and radiographic agent administration[13,14]. Therapeutic doses of hirudin or other direct thrombin inhibitors may prolong clotting times[15].

Lipoglycopeptide antibacterial drugs (such as oritavancin or telavancin) may interfere with APTT based assays. Consult Instructions for Use of respective drugs.

In addition, the choice of anticoagulant (i.e. citrate vs. oxalate) and the condition of the specimen (e.g. hemolyzed, lipemic, parenteral feeding, etc.) may affect results[7,16,17].

The latter is particularly true of optical instrumentation measurements of the APTT. Blood clotting factor deficiencies which should produce prolonged clotting times may be compensated for or made to appear normal by elevated levels of one or more different clotting factors. Similarly, the presence of active intermediates which would tend to reduce the clotting time may also mask conditions that would normally lead to prolongation of the APTT. Mild or minor deficiencies in several factors may have an additive effect on increasing the APTT. ACTIN FS may provide variable APTT results in samples containing the lupus-like anticoagulant.

Unexpected abnormal APTT results should always be followed by additional coagulation studies to determine the source of abnormal results.

Action of heparin as an anticoagulant is related to its ability in conjunction with a plasma cofactor to interfere with several aspects of the coagulation mechanism, thus retarding the rate of fibrin formation (see Section "Monitoring of Unfractionated Heparin Therapy with APTT", page 4).

Siemens Healthineers has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens Healthineers as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Healthineers Reference Guides (Application Sheets) or these Instructions for Use.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Expected Values

In a study of ostensibly healthy individuals using a specific lot of ACTIN FS, the following values were obtained:

			90 % Reference Interval	
	n	Median (s)	5th Percentile (s)	95th Percentile (s)
SYSMEX CA-1500 System	111	25.1	22.1	28.1
BCS® System	111	26.8	23.0	31.9

Reference ranges for other populations such as pediatric groups should also be established where warranted.

Reference intervals vary from laboratory to laboratory depending on the population served and the technique, method, equipment and reagent lot used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the aforementioned variables are changed.

Note: CLSI Document C28-A2 (cited in H47-A)[20,21] states that a parametric approach (mean ±2 SD) can be applied. The assumption of this approach (Gaussian normal distribution) must however be checked.

Performance Characteristics

Precision

Precision studies using the methodologies listed in this insert show that properly performed APTT tests should result in a standard deviation (SD) which corresponds to a coefficient of variation (CV) of less than 4 % in the normal range. In additional clinical studies, duplicate determinations of an abnormal control plasma (clotting times of approximately 50 s) were performed over a period of 22 days. Results indicate that the APTT should agree within 4 % when performed properly.

Technical Assistance

For customer support, contact your local technical support provider or distributor.

siemens-healthineers.com

Current Version of Application Sheets

ACTIN FS can be used in combination with various automated coagulation analyzers.
Siemens Healthineers provides Reference Guides/Application Sheets for the coagulation analyzers listed in section "Materials Required but not Provided", page 3 under the dedicated link below:

siemens-healthineers.com/rg

As Siemens Healthineers continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Siemens Healthineers ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via siemens-healthineers.com/eIFU.

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Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community		Contains sufficient for <n> tests
	Biological Risks	IVD	*In Vitro* Diagnostic Medical Device
	Temperature Limitation		Consult instruction for Use
	Non-sterile		CE marking of conformity
CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.	CONTENTS	Contents
	Reconstitution volume	LEVEL	Level
	Keep away from sunlight and heat	WARNING	Warning
DANGER	Danger	RxOnly	Prescription device (US only)
UDI	Device Identification (UDI) barcode	REACH xx/xx/xx	REACH Authorization Number

Legal Information

(The values stated for the BCS® System do not take into account the correction factors used in the USA.)

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