Berichrom® Plasminogen
Berichrom PLASMINOGEN

Revision bar indicates update to previous version.

CE 0197

Reagents for the determination of plasminogen

Intended Use

Berichrom PLASMINOGEN is an in vitro diagnostic reagent for the quantitative determination of plasminogen activity as aid to diagnosis and monitoring of congenital or acquired plasminogen deficiencies in patients at risk for or suspected to have disorders of the fibrinolysis system in human sodium citrated plasma by means of automated chromogenic methods.

For determination of plasminogen, no international reference preparation or method is available.

Summary and Explanation

Plasminogen, an 81 kDa glycoprotein built in the liver, is the proenzyme of the proteolytic enzyme plasmin which is the main enzyme responsible for the dissolution of fibrin clots, but also can degrade fibrinogen. Physiological plasmin generation is induced via proteolytic activation by endogenous activators such as t-PA (tissue-type plasminogen activator) or u-PA (urokinase-type plasminogen activator). An accelerated plasmin activation can be induced by application of thrombolytic therapy. Plasmin is responsible for limiting the extent of the hemostatic process at the site of vessel injury[1].

Deficiency of plasminogen may be inherited or acquired. Congenital plasminogen deficiency (quantitative or qualitative) is a rare autosomal transmitted disorder, going along with an increased risk for development of an ocular complication called ligneous conjunctivitis. However, decreased plasminogen concentrations can also result from diminished synthesis (liver disease) or increased consumption, e.g. disseminated intravascular coagulation (DIC), and thrombolytic therapy[1–3].

Principles of the Procedure

The plasminogen in the sample forms a complex with the streptokinase in the cuvette/tube. The concentration of this complex is determined in a kinetic test by measuring the increase in absorbance at 405 nm. The test is based on the following reactions.

$$\text{plasminogen} + \text{streptokinase} \longrightarrow [\text{plasminogen-streptokinase}]$$

$$\text{HD-Nva-CHA-Lys-pNA} \xrightarrow{[\text{plasminogen-streptokinase}]} \text{HD-Nva-CHA-Lys-OH} + \text{p-nitroaniline}$$

Reagents

Note: Berichrom PLASMINOGEN can be used manually or on automated coagulation analyzers. Sysmex 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
Berichrom® Plasminogen Berichrom PLASMINOGEN			

REAGENT STR	Lyophilized reagent containing:
	• streptokinase (~0.5 µmol/L)
	• Stabilizer:
	  • Phosphate buffer
	  • Polygeline
	• Preservative:
	  • Sodium azide (reconstituted: < 1 g/L)
		2–8 °C
		May be used up to the expiry date indicated on the label if stored unopened.
			37 °C: reconstituted, 1 week;
			30 °C: reconstituted, 2 weeks;
			25 °C: reconstituted, 2 weeks;
			2–8 °C: reconstituted, 4 weeks;
			≤ –20 °C: reconstituted, 6 months

PLASMIN SUBSTRATE	Lyophilized reagent containing: • HD-Nva-CHA-Lys-pNA (reconstituted: 3 mmol/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	37 °C: reconstituted, 1 week; 30 °C: reconstituted, 2 weeks; 25 °C: reconstituted, 2 weeks; 2–8 °C: reconstituted, 6 weeks; ≤ –20 °C: reconstituted, 6 months

In the original vials dissolved REAGENT STR and dissolved PLASMIN SUBSTRATE can be frozen up to 10 times.

On-board stability
Information about 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.

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State through your local distribution representative in which the user and/or patient is established.

Safety data sheets (MSDS/SDS) available upon request.

Danger! Berichrom PLASMINOGEN REAGENT STR
Hazardous ingredient: Sodium azide (5.34 % [w/w]).
H311: Toxic in contact with skin. **H302**: Harmful if swallowed. **H411**: Toxic to aquatic life with long lasting effects.
P264: Wash hands thoroughly after handling. **P280**: Wear protective gloves/protective clothing/eye protection/face protection. **P273**: Avoid release to the environment. **P391**: Collect spillage. **P312**: Call a POISON CENTER or doctor/physician if you feel unwell. **P361 + P364**: Take off immediately all contaminated clothing and wash it before reuse. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.

Berichrom PLASMINOGEN REAGENT STR
Hazardous ingredient: streptokinase.
May produce an allergic reaction.

Caution
Berichrom PLASMINOGEN REAGENT STR
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

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

REAGENT STR: Dissolve in the labelled quantity of distilled water.

PLASMIN SUBSTRATE: Dissolve vial contents with the labelled quantity of distilled water. Mix carefully once more before using.

Specimen Collection and Handling

Collecting the Specimen

To obtain the plasma, carefully mix 1 part sodium citrate solution (0.11 mol/L (3.2 %)) with 9 parts venous blood, avoiding the formation of foam. Centrifuge the blood specimen immediately at 1 500 × g for no less than 15 minutes at room temperature. Please refer to CLSI guideline H21-A5[4] for further details.

Storing the Specimen

Stability of the samples:

≤ −20 °C	1 month
2 to 8 °C	2 days
15 to 25 °C	4 hours

Plasma stored at ≤ −20 °C is to be thawed within 10 minutes at 37 °C after which the assay is to be performed within 2 hours. Do not freeze repeatedly.

Procedure

Materials Provided

REF	Contents	
OUCA17	Berichrom® Plasminogen Berichrom PLASMINOGEN	
	Streptokinase Reagent Berichrom PLASMINOGEN REAGENT STR	3 × → 5 mL
	Plasmin Substrate Berichrom PLASMINOGEN PLASMIN SUBSTRATE	3 × → 2 mL

Materials Required but not Provided

Item	Description
REF ORKL17	STANDARD PLASMA, Standard Human Plasma
REF ORKE41	CONTROL N, Control Plasma N
REF OUPZ17	CONTROL P, Control Plasma P
–	Acetic acid 20 % (only for the two-point method)
REF B4234-25 REF B4265-37 –	OV BUFFER, Dade® Owren's Veronal Buffer, or CA SYSTEM BUFFER, Dade® CA System Buffer, or Isotonic Saline Solution
Coagulation analyzers[a], such as:	• AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System) • AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System)

[a] Availability of analyzers may vary by country.

Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified.

Test Procedure

Manual:
Semi-micro test:
Cuvette: 1 cm path length
Wavelength: 405 nm
Test temperature: 37 °C
Pre-warm REAGENT STR and PLASMIN SUBSTRATE as well as the plastic cuvettes/tubes to the chosen test temperature.

Pipetting scheme for the kinetic method

Plasma sample	20 µL
REAGENT STR	1000 µL
Mix and incubate for 5 minutes at 37 °C.	
PLASMIN SUBSTRATE	100 µL
Mix and determine $\Delta A_{405\ nm}$/min Photometer/stopwatch: Read the absorbance within 30 seconds and start the stopwatch simultaneously. After exactly 60 and 120 seconds read the absorbance again, calculate the respective ΔA/min values by subtraction and then calculate the mean value of the two ΔA readings.	

Evaluation, kinetic method
Each assay series requires at least 1 reference measurement value, which is determined using a reference plasma with an assigned value for plasminogen in % of Norm (e.g. STANDARD PLASMA). This value is used to calculate the laboratory-internal factor F_L:

$$F_L = \frac{\text{Assigned value (\% of Norm)}_{\text{reference plasma}}}{\Delta A/\text{min}_{\text{reference plasma}}}$$

The plasminogen content of the sample in % of Norm can then be calculated from the following formula:

$\text{plasminogen}_{\text{sample}}$ (% of Norm) = F_L x $\Delta A/\text{min}_{\text{sample}}$

Pipetting scheme for the two-point method

	Sample	Sample blank
Isotonic saline	–	1 000 µL
Plasma sample	20 µL	20 µL
REAGENT STR	1 000 µL	–
Mix and incubate for 5 minutes at 37 °C.		
PLASMIN SUBSTRATE	100 µL	100 µL
Mix immediately and start the stopwatch simultaneously. After exactly 2 minutes, add:		
Acetic acid 20 %	500 µL	500 µL
Mix immediately and read the absorbance against the sample blank within 60 minutes.		

Evaluation, two-point method

Each assay series requires at least 1 reference measurement value, which is determined using a reference plasma with an assigned value for plasminogen in % of Norm (e.g. Standard Human Plasma). This value is used to calculate the laboratory-internal factor F_L:

$$F_L = \frac{\text{Assigned value (\% of Norm)}_{\text{reference plasma}}}{A_{\text{reference plasma}}}$$

The plasminogen content of the sample in % of Norm can then be calculated from the following formula:

$\text{plasminogen}_{\text{sample}}$ (% of Norm) = F_L x A_{sample}

Notes

1. Doubling or halving the volumes used in the test mixtures has no effect on the calculation or laboratory-internal calculation factor.
2. If the sample has a plasminogen content below 20 % of Norm, it is recommended to repeat the test using 40 µL sample. The result is divided by 2.
3. The laboratory-internal factor F_L and/or reference curve must be re-determined for each change of device and for each new lot of Berichrom PLASMINOGEN.

Internal Quality Control

Normal range: CONTROL N

Pathological range: CONTROL P

Two controls should be measured with each calibration and at least every 8 hours during each work day (one in the normal range and one in the pathological range). The controls should be processed just like the samples. Each laboratory should determine its own quality control range, either by means of the target values and ranges provided by the manufacturer of the controls or by means of its own confidence range established in the laboratory. If the measured control value lies outside the confidence range previously established, then the reagents, the laboratory-internal factor F_L and/or the reference curve and coagulation analyzer should be examined. Do not release patient results until the cause of deviation has been identified and corrected.

Limitations

Falsely low plasminogen activities may be obtained in patients undergoing treatment with aprotinin[5,6].

This procedure measures biological activity of plasminogen rather than immunoreactive concentration of plasminogen. If all plasminogen molecules in the sample are not biologically active, concentrations measured by the two methodologies will not be equivalent.

Failure to perform the assay at a constant temperature will invalidate results.

The manufacturer has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified. User defined modifications are not supported by the manufacturer 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 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

75 - 150 % of Norm[7].

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.

Performance Characteristics

Measuring Range

The generic measuring range extends from 0 to 150 % of Norm.

The measuring range depends on the individual application of the assay due to instrument related conditions. Application specific performance data is listed in the respective Reference Guides of the instruments.

Precision

Within-series coefficients of variation (CV) for a sample in the normal range was 2.9 % while, in the pathological range, the CV was 4.0 %. Similarly, the inter-assay reproducibility was 3.6 % for normal and 5.3 % for pathological samples, respectively.

The reproducibility was assessed by the manufacturer for Plasminogen assay with Berichrom PLASMINOGEN based on proficiency testing information in 2020. The overall reproducibility median CV% was found to be <10 % including lot, instrument, laboratory, and operator variability factors.

Method Comparison

In a comparison of Berichrom PLASMINOGEN with other chromogenic methods, the correlation coefficients found were between 0.88 (two-point method) and 0.99 (kinetic method).

Technical Assistance

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

Current Version of Application Sheets

Berichrom PLASMINOGEN can be used in combination with various automated coagulation analyzers. Sysmex provides Reference Guides/Application Sheets for the coagulation analyzers listed in section "Materials Required but not Provided", page 4 under the dedicated link below:

sysmex-ifu.com/ag

As the manufacturer 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. Sysmex ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via sysmex-ifu.com.

References

1. Mehta R, Shapiro AD. Plasminogen deficiency. Haemophilia 2008;14(6):1261-8.
2. Schuster V, Hügle B, Tefs K. Plasminogen deficiency. J Thromb Haemost. 2007;5(12):2315-22.
3. Celkan T. Plasminogen deficiency. J Thromb Thrombolysis 2017;43(1):132-138.
4. CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays. Approved Guideline – Fifth Edition. CLSI document **H21-A5** [ISBN 1-56238-657-3]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
5. Wendel HP, Heller W, Gallimore MJ. Aprotinin in therapeutic doses inhibits chromogenic peptide substrate assays for protein C. Thromb Res. 1994;74:543-8.
6. Thomas L. Clinical Laboratory Diagnostics, TH-Books Verlagsgesellschaft mbH, Frankfurt/ Main, Germany, 1998: 607.
7. Kraus M. Plasminogen. In: Thomas L, ed. Clinical Laboratory Diagnostics, 5th ed. Frankfurt: TH-Books Verlagsgesellschaft, 1998: 625-7.

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	CH REP	Authorized representative in Switzerland
Σ	Contains sufficient for <n> tests		Biological Risks
IVD	*In Vitro* Diagnostic Medical Device		Temperature Limitation
	Consult instruction for Use	NON STERILE	Non-sterile
CE	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

Siemens Healthcare Diagnostics Products GmbH
Emil-von-Behring-Str. 76
35041 Marburg
Germany
siemens-healthineers.com