# Berichrom® Antithrombin III (A)
# Berichrom AT III

Revision bar indicates update to previous version.

CE 0197

## Intended Use

Berichrom AT III is an in vitro diagnostic reagent for the quantitative, WHO-standardized determination of antithrombin as aid to diagnosis and monitoring of congenital or acquired antithrombin deficiencies in patients at risk for or suspected to have antithrombin deficiency in human sodium citrated plasma by means of automated, chromogenic methods. In addition Berichrom AT III assay can be used for monitoring antithrombin substitution therapy.

## Summary and Explanation

Antithrombin (AT) is a natural anticoagulant that circulates in the plasma at a concentration of 112 to 140 mg/L with a half-life of 2 to 3 days. It is a serine protease inhibitor (serpin) which irreversibly inhibits not only thrombin and FXa, but also FIXa, FXIa, FXIIa, kallikrein, and plasmin[1,2]. The inhibition of thrombin and FXa by AT is accelerated approximately one thousand-fold in the presence of heparan sulfate (in vivo), or heparin.

Genetically caused AT deficiency is associated with a high risk for thromboembolic events, which typically manifest at young age <40 years. Hereditary AT deficiency is classified in type I and type II deficiency states, where type I (quantitative) deficiency is typically caused by reduced secretion of functionally normal AT into the blood, whereas type II (qualitative) deficiencies are qualitative defects resulting in the production of a variant protein with decreased function.

AT is synthesized in the liver; acquired AT deficiency can result from reduced synthesis, increased protein consumption, or as a consequence of protein loss in conditions such as DIC (disseminated intravascular coagulation), sepsis, acute hemolytic transfusion reaction, increased protein loss in nephrotic syndrome, thrombotic microangiopathies, malignant diseases, acute thrombotic episodes and asparaginase therapy. Acquired deficiencies of AT are associated with hypercoagulable or consumptive states[1,2]. For investigation of thrombophilic patients with suspected hereditary or acquired AT deficiency, a chromogenic activity assay such as Berichrom AT III is recommended[3–5].

## Principles of the Procedure

The Antithrombin III (ATIII) in the sample is converted by heparin into an immediate inhibitor and inactivates the thrombin present. The residual thrombin content is determined in a kinetic test measuring the increase in absorbance at 405 nm according to the following reaction:

$$\text{AT III}_{\text{sample}} + \text{Thrombin}_{\text{excess}} \xrightarrow{\text{Heparin}} [\text{AT III-Thrombin}] + \text{Thrombin}_{\text{residual}}$$

$$\text{Tos-Gly-Pro-Arg-ANBA-IPA} \xrightarrow{\text{residual thrombin}} \text{Tos-Gly-Pro-Arg-OH} + \text{ANBA-IPA}$$

The absorbance change is inversely correlated to the Antithrombin III activity in the Sample.

# Reagents

**Note:** Berichrom AT III 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® Antithrombin III (A)<br>Berichrom AT III | | | |
| REAGENT THR | Lyophilized reagent containing:<br>• Thrombin, bovine<br>• Heparin sodium, porcine<br>• Aprotinin | 2–8 °C<br>May be used up to the expiry date indicated on the label if stored unopened. | 2–8 °C: reconstituted, 2 weeks[a];<br>–20 °C: reconstituted, 3 months[a] |
| SUBSTRATE | Lyophilized reagent containing:<br>• Tos-Gly-Pro-Arg-ANBA-IPA (reconstituted: 4 mmol/L[b]) | 2–8 °C<br>May be used up to the expiry date indicated on the label if stored unopened. | 2–8 °C: reconstituted, 6 weeks[a];<br>–20 °C: reconstituted, 6 months[a] |
| REAGENT THR DILUENT | Ready to use liquid containing:<br>• TRIS/HCl (100 mmol/L)<br>• NaCl (8.7 g/L)<br>• Preservative:<br>  • Sodium azide (<1 g/L) | 2–8 °C<br>May be used up to the expiry date indicated on the label if stored unopened. | 2–8 °C: once opened, 6 months[a] |

[a] closed original vial
[b] concentration in the working solution

Reconstituted REAGENT THR may be frozen and thawed in the original vial up to five times.

Reconstituted SUBSTRATE may be frozen and thawed in the original vial up to ten times.

Do not exceed the stability period listed in the table.

**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.

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.

**Berichrom AT III REAGENT THR**
Hazardous ingredient: Thrombin, bovine (0.233 % [w/w]).
May produce an allergic reaction.

**Caution**
**Berichrom AT III REAGENT THR**
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.

Summary of Safety and Performance (SSP) is available in the European database on medical devices (see Eudamed public website: https://ec.europa.eu/tools/eudamed). In case Eudamed is not available, SSP can be delivered by the manufacturer on request.

### Preparing Reagents

| | |
|---|---|
| REAGENT THR: | Reconstitute with the amount of REAGENT THR DILUENT indicated on the label and incubate for 30 minutes at 15 to 25 °C before use.<br>To ensure the homogeneity of the reagent, after reconstitution and shortly before use, gently mix the dissolved reagent. |
| SUBSTRATE: | Dissolve the contents of the vial with the amount of distilled or deionized water indicated on the label. |

**Note:** Make sure that SUBSTRATE is fully dissolved.

## Specimen Collection and Handling

### Collecting the Specimen

To obtain the plasma, carefully mix 1 part sodium citrate solution (0.11 mol/L or 3.2 %) with 9 parts venous blood, avoiding the formation of foam. An evacuated tube system or syringe may be used. Please refer to CLSI document H21-A5 for further details[6].

Centrifuge immediately at no less than 1 500 × g for ≥15 minutes.

### 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 multiple times.

Use the plasma undiluted.

# Procedure

## Materials Provided

| REF | Contents | |
|---|---|---|
| OWWR17 | Berichrom® Antithrombin III (A)<br>Berichrom AT III | |
| | Thrombin Reagent<br>Berichrom AT III REAGENT THR | 6 × → 5 mL |
| | Substrate Reagent<br>Berichrom AT III SUBSTRATE | 3 × → 3 mL |
| | Buffer Solution<br>Berichrom AT III REAGENT THR DILUENT | 1 × 30 mL |
| OWWR15 | Berichrom® Antithrombin III (A)<br>Berichrom AT III | |
| | Thrombin Reagent<br>Berichrom AT III REAGENT THR | 6 × → 15 mL |
| | Substrate Reagent<br>Berichrom AT III SUBSTRATE | 6 × → 3 mL |
| | Buffer Solution<br>Berichrom AT III REAGENT THR DILUENT | 1 × 100 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 |
| REF OQAA33<br>REF B4234-25<br>REF B4265-37<br>– | IMIDAZOLE BUFFER, Imidazole Buffer Solution, or<br>OV BUFFER, Dade® Owren's Veronal Buffer, or<br>CA SYSTEM BUFFER, Dade® CA System Buffer, or<br>Isotonic Saline Solution |
| – | Distilled or deionized water without preservatives |
| Coagulation analyzers[c], such as: | • Automated Blood Coagulation Analyzer CA-600 series (CA-600 series)<br>• AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System)<br>• AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System) |

[c] 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

Pipetting scheme (example)

| | |
|---|---|
| Citrated plasma | 10 µL |
| REAGENT THR | 600 µL |
| | Mix well. Incubate at 37 °C for 3 minutes. |
| SUBSTRATE | 100 µL |
| | Determine $\Delta A_{405\ nm}$ /min |

**Evaluation**
Analysis takes place automatically in the coagulation analyzer.

### Calculating the Reference Curve

A reference curve is generated by automatic determination of different dilutions of STANDARD PLASMA. The reference curve must be re-generated if there is a change in the instrument or in the lot of Berichrom AT III (A) used, or if there is any change in the experimental conditions.

### Internal Quality Control

Normal range: CONTROL N
Pathological range: CONTROL P
Two levels of quality control material (normal and pathologic range) have to be measured at start of the test run, with each calibration, upon reagent vial changes and at least every eight hours on each day of testing. 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 control values determined in the laboratory. If the measured control value lies outside of the pre-determined range, then the reagents, the reference curve and the coagulation analyzer should be checked. Do not report patient results until the problem has been identified, corrected and documented.

## Limitations

Therapeutic doses of hirudin or other direct thrombin inhibitors will cause erroneously increased ATIII activity.

Some very rare genetic variants with reduced functional activity like ATIII Basel may yield results within the reference range.

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

In a study of ostensibly healthy individuals using a specific lot of Berichrom AT III, the following values were obtained:

| | | | % Reference Interval | |
|---|---|---|---|---|
| | n | Median [% of Norm] | 2.5th Percentile [% of Norm][d] | 97.5th Percentile [% of Norm][d] |
| BCS® System/BCS® XP System | 309 | – | 79.4 | 112 |

[d] For adults

Other system specific results are given in the respective Reference Guides (Application Sheets).

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 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 Guide of the instrument.

## Specificity

Aprotinin in the Thrombin Reagent blocks the activity of any plasmin present in the sample[7].

Interference from heparin cofactor II can be disregarded since bovine thrombin is used in the test[8].

Contraceptives under hormone replacement therapy could decrease the AT concentration in plasma of 10 %[9].

Oral anticoagulant therapy with vitamin K antagonists such as warfarin or coumarin may lead to an increase in AT activity[10].

## Sensitivity

The Limit of Quantitation (LoQ) of the assay Antithrombin III/ Berichrom AT III depends on the instrument on which the assay is applied and was determined in the range of 2.6 to 9.4 % of Norm.

## Precision

The precision of Berichrom AT III was calculated with CONTROL N and CONTROL P on a CA-1500 System over 5 days in 8-fold determination.

The coefficient of variation within run was 1.3 % and 2.7 % for CONTROL N and CONTROL P, respectively. From day to day, it was 4.6 % and 7.6 %, respectively.

Other system specific results are given in the respective Reference Guides (Application Sheets).

The reproducibility was assessed by the manufacturer for Berichrom® Antithrombin III (A) based on publicly available proficiency testing information in 2019. The overall reproducibility median CV % was found to be <6 % including lot, instrument, laboratory and operator variability factors.

## Method Comparison

Regression analysis of the results yielded the following equations:

| | n | Slope | Intercept [% of Norm] | Correlation Coefficient |
|---|---|---|---|---|
| Berichrom AT III (A) with Berichrom AT III (with manual dilution) on Hitachi 717 | 111[e] | 0.98 | −3.7 | 0.99 |

[e] range from 25 to 150 % of Norm

# Technical Assistance

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

## Current Version of Application Sheets

Berichrom AT III 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. Maclean PS, Tait RC. Hereditary and acquired antithrombin deficiency: epidemiology, pathogenesis and treatment options. Drugs. 2007;67:1429-40.
2. Kottke-Marchant K, Duncan A. Antithrombin deficiency – issues in laboratory diagnosis. Arch Pathol Lab Med. 2002;126:1326-36.
3. Khor B, Van Cott EM. Laboratory tests for antithrombin deficiency. Am J Hematol. 2010;85:947-50.
4. Luxembourg B, Delev D, Geisen C, et al. Molecular basis of antithrombin deficiency. Thromb Haemost. 2011;105:635-46.
5. Marlar RA, Gausman JN. Laboratory testing issues for protein C, protein S, and antithrombin. Int J Lab Hematol. 2014;36:289-95.
6. 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.
7. Wendel HP, Heller W, Gallimore MJ. Aprotinin in therapeutic doses inhibits chromogenic peptide substrate assays for protein C. Thromb Res. 1994;74:543-8.
8. Friberger P, Egberg N, Holmer E, et al. Antithrombin assay - the use of human or bovine thrombin and the observation of a „second“ heparin cofactor. Thromb Res. 1982; 25: 433-6.
9. Lichte A. Chapter 2.7.1 in: Bruhn HD, Schambeck C, Hach-Wunderle V, editors. Hämostaseologie für die Praxis. Schattauer GmbH; 2007
10. Thomas L., Chapter 16. in: Clinical Laboratory Diagnostics (Labor und Diagnose) [electronic Edition, mobile application form (app)]. TH Books; 2016.


# 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



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