SIEMENS

CE

ADVIA® Chemistry XPT
Systems

Alkaline Phosphatase_2 Concentrated (ALP_2c)

Current Revision and Date[a]	Rev. D, 2020-02	
Product Name	ADVIA® Chemistry Alkaline Phosphatase_2 Concentrated (ALP_2c) Reagents	REF 10916067
Systems	ADVIA Chemistry XPT System	
Materials Required but Not Provided	ADVIA Chemistry Alkaline Phosphatase_2 (ALP_2) Calibrator Reagent container adapters Commercially available controls	REF 10916060
Specimen Types	Human serum, plasma (lithium heparin)	
Assay Principle	IFCC Standardization	
Assay Range	Serum: 10–1000 U/L Plasma: 10–1000 U/L	
Reagent Storage	2–8°C	
Reagent On-System Stability	28 days when you are using a reagent container insert 21 days when you are not using a reagent container insert	
Reagent Code	74031	

[a] In Rev. B or later, a vertical bar in the margin indicates a technical update to the previous version.

Intended Use

For *in vitro* diagnostic use in the quantitative measurement of alkaline phosphatase in human serum or plasma on ADVIA® Chemistry XPT systems. Measurements of alkaline phosphatase or its isoenzymes are used in the diagnosis and treatment of liver, bone, parathyroid, and intestinal diseases.

Summary and Explanation

The ADVIA Chemistry Alkaline Phosphatase_2 Concentrated (ALP_2c) assay is based on the primary reference procedure for the measurement of catalytic activity of alkaline phosphatase at 37°C as described by the International Federation of Clinical Chemistry (IFCC). The alkaline phosphatase method is based on a procedure published by Bowers and McComb[1] and more recently reviewed by Rej.[2] This assay responds to all alkaline phosphatase isoenzymes in human serum.[3]

Principles of the Procedure

Alkaline phosphatase catalyzes the transphosphorylation of *p*-nitrophenylphosphate (*p*-NPP) to *p*-nitrophenol (*p*-NP) in the presence of the transphosphorylating buffer, 2-amino-2-methyl-1-propanol (AMP). The reaction is enhanced through the use of magnesium and zinc ions. The change in absorbance at 410 nm due to the formation of *p*-NP is directly proportional to the ADVIA Chemistry (ALP_2c) activity, since other reactants are present in non-rate limiting quantities. The change is measured using a bichromatic (410/478 nm) rate technique.

Reaction Equation

$$p\text{-NPP} + \text{AMP} \xrightarrow[\text{pH 10.25}\ \text{Mg/Zn}]{\text{ALP}} p\text{-NP} + \text{AMP} + PO_4$$

Reagents

Reagent	Description	Storage	Reagent Stability
REF 10916067	**ADVIA Chemistry Alkaline Phosphatase_2 Concentrated (ALP_2c) Reagents**		
Alkaline Phosphatase_2 Concentrated Reagent 1 ALP_2c R1	37.6 mL in 40-mL containers 2-amino-2-methyl-1-propanol (AMP) (3.0 mol/L) Hydroxyethyl ethylevediamine triacetic acid (HEDTA) (8.0 mmol/L) Magnesium acetate (8.0 mmol/L) Zinc sulfate (4.0 mmol/L) NaN_3 (0.09%)	2–8°C	**Unopened:** Stable until the expiration date on product. **On-system :** • 28 days when you are using a reagent container insert • 21 days when you are not using a reagent container insert.
Alkaline Phosphatase_2 Concentrated Reagent 2 ALP_2c R2	38.0 mL in 40-mL containers Paranitrophenyl-phosphate (*p*-NPP) substrate (101.6 mmol/L) NaN_3 (0.09%) ProClin 300 (0.024%)	2–8°C	**Unopened:** Stable until the expiration date on product. **On-system :** • 28 days when you are using a reagent container insert • 21 days when you are not using a reagent container insert.

Warnings and Precautions

Safety data sheets (MSDS/SDS) available on siemens.com/healthcare.

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.

H319, H315, H412
P280, P273, P305+P351+P338, P501

Warning!
Causes serious eye irritation. Causes skin irritation. Harmful to aquatic life with long lasting effects.
Wear protective gloves/protective clothing/eye protection/face protection. Avoid release to the environment. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Dispose of contents and container in accordance with all local, regional, and national regulations.
Contains: 2-amino-2-methylpropanol; sulfuric acid, zinc salt (1:1), heptahydrate; ADVIA Chemistry ALP_2c Reagent 1

Contains: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1) (ProClin 300). May produce an allergic reaction. ADVIA Chemistry ALP_2c Reagent 2.

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 prevailing regulatory requirements.

Caution: Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional.

For *in vitro* diagnostic use.

Preparing Reagents

All reagents are liquid and ready to use.

Before use, gently invert the capped reagent to disrupt bubbles and ensure homogeneity. If bubbles still exist or foam is present, use a clean transfer pipette to aspirate them from the reagent container prior to use.

Storing and Stability

Unopened reagents are stable until the expiration date printed on the product label when stored at 2–8°C. Do not freeze reagents. Protect reagents from light.

Specimen Collection and Handling

Siemens Healthcare Diagnostics has qualified serum and plasma (lithium heparin) for the ADVIA Chemistry ALP_2c assay.

The purpose of handling and storage information is to provide guidance to users. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria to meet specific needs.

Collecting the Specimen

Follow these guidelines for specimens used for this assay:

- Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this assay. In the preparation of serum or plasma samples, avoid prolonged contact with separated red cells.[4]
- Follow the instructions provided with your specimen collection device for use and processing.[5]
- Complete clot formation should take place before centrifugation.
- Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of 2 hours from the time of collection.[6]
- Thawed specimens that are turbid must be clarified by centrifugation prior to testing.

Storing the Specimen

Specimens are stable under the following conditions:[7, 8]

- For 8 hours at room temperature
- For 7 days at 2–8°C
- For 6 months when frozen at -20°C or colder.

Avoid repeated freezing and thawing.

Procedure

Materials Provided

The following materials are provided:

Item	Contents	Number of Tests
REF 10916067	Reagent 1: 6 × 40-mL containers Reagent 2: 6 × 40-mL containers	6 × 1240

Materials Required but Not Provided

The following materials are required to perform this assay, but are not provided:

Item	Description
REF 10916060	ADVIA Chemistry Alkaline Phosphatase_2 (ALP_2) Calibrator
REF 10719152	40-mL reagent container adapter for 70-mL slot
	Commercially available control materials

Optional Materials

The following materials may be used to perform this assay, but are not provided:

Item	Description
REF 02991886	Reagent container insert

Assay Procedure

For detailed instructions on performing the procedure, refer to the system operating instructions.

The ADVIA Chemistry XPT system automatically performs the following actions:

1. Dispenses Reagent 1 into a cuvette
2. Adds the sample into the same cuvette and mixes the contents
3. Adds Reagent 2 into the same cuvette and mixes the contents
4. Adds water as a diluent into the cuvette
5. Incubates the cuvette for 5 minutes at 37°C
6. Reports results as a rate reaction measured at 410 and 478 nm.

Reaction time: 10 minutes

Preparing the System

Sampling, reagent delivery, mixing, and processing are automatically performed by the ADVIA Chemistry system.

For detailed information on preparing the system, refer to the system operating instructions.

Preparing the Samples

This assay requires 9.4 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume that is required when you are performing duplicates or other tests on the same sample. For detailed information about how to determine the minimum required sample volume, refer to the system operating instructions.

Before placing samples on the system, ensure that samples have the following characteristics:

- Samples are free of fibrin or other particulate matter.
- Samples are free of bubbles.

On-System Stability

The ADVIA Chemistry ALP_2c reagents are stable on the system for 28 days when you are using a reagent container insert, and for 21 days when you are not using a reagent container insert.

Do not use reagents beyond the expiration date.

Performing Calibration

To calibrate the ADVIA Chemistry ALP_2c assay, use the ADVIA Chemistry Alkaline Phosphatase_2 (ALP_2) Calibrator, REF 10916060.

Enter the lot-specific calibrator values that are provided with each lot of calibrator. Perform the calibration as described in the calibrator instructions for use.

Calibration Frequency

Calibrate the assay every 28 days when you are using a reagent container insert, and every 21 days when you are not using a reagent container insert.

Calibrate the assay after the following events:

- When the reagent lot number changes
- When a reagent pack is replaced by a new reagent pack with the same lot number, and the previous reagent pack was recalibrated during use
- When a reagent pack is replaced by a new reagent pack with the same lot number, and an additional reagent blank was run on the previous reagent pack during use
- After replacing critical optical or hydraulic components
- When indicated by quality control procedures

Individual laboratory quality control programs and procedures may require more frequent calibration.

Reagent Blank (RBL) Frequency

The ADVIA Chemistry system measures the RBL during assay calibration.

Note Use deionized water or isotonic saline solution as the sample for the RBL in the ADVIA Chemistry ALP_2c assay.

Performing Quality Control

Follow government regulations or accreditation requirements for quality control frequency.

At least once each day of use, analyze 2 levels (low and high) of a commercially available quality control (QC) material with known alkaline phosphatase concentrations.

A satisfactory level of performance is achieved when the analyte values obtained are within the expected control range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme.

The actual frequency of control in a laboratory is based on many factors, such as workflow, system experience, and government regulation. Each laboratory should evaluate the controls based on the frequency established by their laboratory guidelines.

Also, assay controls under the following conditions:

- Whenever you use a new reagent lot
- Following any system maintenance, cleaning, or troubleshooting procedure
- After performing a new calibration or an additional reagent blank

Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.

Taking Corrective Action

If the quality control results do not fall within the expected control range or within the laboratory's established values, do not report results. Take the following actions:

1. Determine and correct the cause of the unacceptable control results:
    a. Verify that the assay was performed according to the instructions for use.
    b. Verify that the materials are not expired.
    c. Verify that required maintenance was performed.
    d. Rerun the assay with fresh quality control samples, and confirm that quality control results are within acceptable limits before running patient samples.
    e. If the quality control results are not within acceptable limits, recalibrate the assay, and repeat the prior step.
    f. If necessary, contact your local technical support provider or distributor for assistance.
2. After corrective action is complete, repeat required testing of patient samples before reporting results.

Perform corrective actions in accordance with your established laboratory protocol.

Results

Calculation of Results

The instrument calculates the concentration of alkaline phosphatase in U/L.

Interpretation of Results

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

Limitations

A number of substances cause physiological changes in serum or plasma analyte concentrations. A comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. Consult the listed reference for specific details on known potential interfering substances.[9]

As with any chemical reaction, you must be alert to the possible effect on results of unknown interferences from medications or endogenous substances. The laboratory and physician must evaluate all patient results in light of the total clinical status of the patient.

Note Siemens has determined that there is a possibility for certain ADVIA Chemistry reagents to interact with the ADVIA Chemistry ALP_2c assay when run on the same system. To mitigate these carryover events, the ADVIA Chemistry system software provides a Contamination Avoidance process. For further information and instructions to establish this process on your systems, refer to the Customer Bulletin entitled: *Consolidated Directory of Contamination Avoidance Settings for ADVIA Chemistry XPT Systems* (PN 10815606, latest revision).

Expected Values

The reference range for alkaline phosphatase is 46–116 U/L.[10]

The reference interval was calculated non-parametrically and represents the central 95% of results determined from a population of healthy adults (n = 132). Results are from the comparative system using the same reagent, with assay conditions identical to those on the ADVIA Chemistry systems.[11]

Siemens provides this information for reference. As with all *in vitro* diagnostic assays, each laboratory should determine its own reference ranges for the diagnostic evaluation of patient results. Consider these values as a guideline only.

Performance Characteristics

Analytical Measuring Range

This assay is linear from 10–1000 U/L.

Results that are below the low end of the assay range are flagged **< Conc Range**. You should report the test result as < 10 U/L.

Results that are above the high end of the assay range are flagged **> Conc Range**.

Extended Measuring Interval

Siemens has qualified an automatic rerun condition for this assay that extends the reportable range to 3800 U/L. You may configure the system to trigger automatic reruns. Rerun results will be flagged **Autorepeat**.

Detection Capability

The assay is designed to have the following characteristics:

- A limit of blank (LoB) < 10 U/L
- A limit of detection (LoD) ≤ 10 U/L
- A limit of quantitation (LoQ) ≤ 11 U/L

The LoB, LoD, and LoQ were determined as described in CLSI Document EP17-A2.[12]

The LoB is the highest measurement result that is likely to be observed on a blank sample. The LoB for the ADVIA Chemistry ALP_2c assay is 0 U/L (rounded to reportable digits).

The LoD is the smallest amount that this assay can reliably detect to determine presence or absence of an analyte. The LoD for the ADVIA Chemistry ALP_2c assay is 2 U/L.

The LoB and LoD values are determined with proportions of false positives (α) less than 5% and false negatives (β) less than 5%, based on 360 determinations with 180 blank and 180 low-level sample replicates.

The LoQ is the lowest amount of alkaline phosphatase that can be determined quantitatively within a defined total error. The LoQ for the ADVIA Chemistry ALP_2c assay is 10 U/L.

Assay results obtained at individual laboratories may vary from the data presented.

Precision

The Repeatability precision of the ADVIA Chemistry ALP_2c assay is designed to have the following characteristics:

- ≤ 3.2% at 50–150 U/L
- ≤ 3.0% at 150–500 U/L
- ≤ 3.0% at 750–950 U/L

The Within-Lab precision of the ADVIA Chemistry ALP_2c assay is designed to have the following characteristics:

- ≤ 5.0% at 50–150 U/L
- ≤ 4.0% at 150–500 U/L
- ≤ 4.0% at 750–950 U/L

Each sample was assayed 2 times per run, 2 runs per day, for at least 20 days. The precision of the assay was analyzed as described in CLSI protocol EP05-A2.[13]

			Repeatability (Within-Run)		Within-Lab (Total)	
Specimen Type	N	Mean (U/L)	SD[a] (U/L)	CV[b] (%)	SD (U/L)	CV (%)
Serum Control 1	80	31	0.3	1.1	1.0	3.3
Serum Control 2	80	144	0.5	0.4	2.0	1.4
Serum Control 3	80	273	0.9	0.3	2.9	1.1
Serum Pool 1	80	108	2.2	2.0	2.4	2.3
Serum Pool 2	80	391	4.2	1.1	6.2	1.6
Serum Pool 3	80	863	21.4	2.5	22.1	2.6

[a] SD = standard deviation

[b] CV = coefficient of variation

Assay results obtained at individual laboratories may vary from the data presented.

Method Comparison

The ADVIA Chemistry ALP_2c assay (y) is designed to have a correlation coefficient ≥ 0.96, when compared to the performance of a comparison assay on the indicated system (x).

The correlation of the assay may vary depending on the study design, comparable method, and sample population. Results obtained at individual laboratories may vary from the data provided.

Specimen Type	Comparison Assay (x)	N	r	Regression Equation	Sy.x	Sample Range
Serum	ADVIA 1800 XXXX	135	0.999	y = 1.03x - 6.12 U/L	12.4 U/L	13–958 U/L
Plasma (Lithium Heparin) ADVIA 1800[a]	Siemens Dimension ALPI	122	0.999	y = 1.03x + 4.37 U/L	9.0 U/L	21–829 U/L

[a] Results are from the ADVIA 1800 Chemistry system using the same reagent, with assay conditions identical to those on the ADVIA Chemistry XPT system.

Assay results obtained at individual laboratories may vary from the data presented.

Interferences

Potential interference in the ADVIA Chemistry ALP_2c assay from bilirubin, hemoglobin, and lipemia is designed to be < 10%. Interference from these substances was tested as described in CLSI protocol EP7-A2 using the ADVIA Chemistry ALP_2c assay.

Interferent	Interferent Level	Alkaline Phosphatase Sample Concentration	Interference
Bilirubin (conjugated)	60 mg/dL	111 U/L	NSI[a]
	60 mg/dL	413 U/L	NSI
	60 mg/dL	905 U/L	NSI
Bilirubin (unconjugated)	60 mg/dL	110 U/L	NSI
	60 mg/dL	410 U/L	NSI
	60 mg/dL	880 U/L	NSI
Hemolysis (hemoglobin)	250 mg/dL	114 U/L	NSI
	500 mg/dL	418 U/L	NSI
	500 mg/dL	939 U/L	NSI
Lipemia (triglycerides from Intralipid)	2000 mg/dL	108 U/L	NSI
	2000 mg/dL	393 U/L	NSI
	1500 mg/dL	857 U/L	NSI

[a] NSI = No significant interference. A percentage effect ≥ 10% is considered a significant interference.

Assay results obtained at individual laboratories may vary from the data presented.

Standardization

The ADVIA Chemistry ALP_2c assay is traceable to the IFCC reference method via patient sample correlation.[10] Assigned values of the ADVIA Chemistry ALP_2 Calibrator are traceable to this standardization.

Technical Assistance

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

siemens.com/healthcare

References

1. Bowers GN, McComb RB. A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. *Clin Chem.* 1966;12:70.
2. Rej R. Effect of incubation with Mg++ on the measurement of alkaline phosphatase activity. *Clin Chem.* 1977; 23:1903–1911.
3. Schumann G, Bonora R, Ceriotti F, Ferard G, Ferrero CA, Franck PF, *et al.* IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 9: Reference procedure for the measurement of catalytic concentration of alkaline chosphatase. *Clin Chem Lab Med.* 2011;49:1439-1446
4. Clinical and Laboratory Standards Institute (formerly NCCLS). *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Guideline—Sixth Edition.* CLSI document GP41-A6. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
5. Clinical and Laboratory Standards Institute (formerly NCCLS). *Tubes and Additives for Venous Blood Specimen Collection: Approved Standard; Approved Guideline—Sixth Edition.* CLSI document GP39-A6. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
6. Clinical and Laboratory Standards Institute (formerly NCCLS). *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Fourth Edition.* CLSI document GP44-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
7. Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 3rd ed. Washington, DC: AACC Press; 2007: 87.
8. Wu AHB, ed. *Tietz Clinical Guide to Laboratory Tests*, 4th edition. St. Louis, MO: WB Saunders Company; 2006:80.
9. Young DS. *Effects of Drugs on Clinical Laboratory Tests.* 5th ed. Washington, DC: AACC Press; 2000.
10. Data on file at Siemens Healthcare Diagnostics.
11. Testing performed on Dimension Clinical Chemistry system; data on file at Siemens Healthcare Diagnostics.
12. Clinical and Laboratory Standards Institute (formerly NCCLS). *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition.* CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
13. Clinical and Laboratory Standards Institute (formerly NCCLS). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition.* CLSI document EP05-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2004.

Definition of Symbols

The following symbols may appear on the product labeling:

Symbol	Definition	Symbol	Definition
IVD	*In vitro* diagnostic medical device	REF REF	Catalog number
	Legal manufacturer	EC REP	Authorized Representative in the European Community
CE	CE Mark	CE 0088	CE Mark with identification number of notified body
	Consult instructions for use		Biological risk
	Keep away from sunlight and heat		Temperature limitation
	Lower limit of temperature		Upper limit of temperature
	Do not freeze (> 0°C)		Up
	Use by	Σ (n)	Contains sufficient for (n) tests
	Recycle	PRINTED WITH SOY INK	Printed with soy ink
Rev.	Revision	YYYY-MM-DD	Date format (year-month-day)
LOT	Batch code	**RxOnly**	Prescription Device (US only)

Trademarks

Siemens Healthcare Diagnostics Inc.
511 Benedict Avenue
Tarrytown, NY 10591 USA

Global Siemens Headquarters
Siemens AG
Wittelsbacherplatz 2
80333 Muenchen
Germany

Global Siemens Healthcare Headquarters
Siemens AG
Healthcare Sector
Henkestrasse 127
91052 Erlangen
Germany
Phone: +49 9131 84-0
siemens.com/healthcare

Global Division
Siemens Healthcare Diagnostics Inc.
511 Benedict Avenue
Tarrytown, NY 10591
USA
siemens.com/healthcare