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ADVIA® 1800
ADVIA® 2400
Chemistry Systems

Alanine Aminotransferase (P5P), Concentrated (ALTPLc)

Current Revision and Date[a]	Rev. C, 2019-11	
Product Name	ADVIA® Chemistry Alanine Aminotransferase Concentrated (ALT_c) Reagents	REF 06860469
	ADVIA® Chemistry Pyridoxal-5'-Phosphate Liquid (P5P_L) Reagent	REF 10729782
Systems	ADVIA Chemistry 1800 System ADVIA Chemistry 2400 System	
Materials Required but Not Provided	ADVIA Chemistry Enzyme 2 Calibrator Reagent container adapters Commercially available controls	REF 10916058
Specimen Types	Human serum, plasma (lithium heparin)	
Assay Principle	Modified IFCC	
Assay Range	Serum: 9–1000 U/L Plasma: 9–1000 U/L	
Reagent Storage	2–8°C	
Reagent On-System Stability	28 days	
Reagent Code	74708	

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

Intended Use

The ADVIA® Chemistry Alanine Aminotransferase P5P (ALTPLc), concentrated assay is for *in vitro* diagnostic use in the quantitative determination of alanine aminotransferase activity in human serum and plasma (lithium heparin) on ADVIA Chemistry systems. This assay is used to aid in the diagnosis and treatment of certain types of liver disease.

Summary and Explanation

The ADVIA Chemistry Alanine Aminotransferase (P5P) Concentrated (ALTPLc) assay uses liquid pyridoxal-5'-phosphate (P5P_L) added to Reagent 1 of the ADVIA Chemistry Alanine Aminotransferase Concentrated (ALT_c) reagent. The assay is based on work by Wroblewski and LaDue, which was later modified by H.U. Bergmeyer. The ADVIA Chemistry ALTPLc reagents are formulated in accordance with the International Federation of Clinical Chemistry (IFCC) recommendations.[1]

Principles of the Procedure

The reaction is initiated by the addition of α-ketoglutarate as a second reagent. The concentration of reduced nicotinamide adenine dinucleotide (NADH) is measured by its absorbance at 340/410 nm and the rate of absorbance decrease is proportional to the alanine aminotransferase (ALT) activity.

Reaction Equation

$$\text{ALT} + \text{P5P} \longrightarrow \text{Activated ALT}$$

$$\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{L-Glutamate}$$

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{Lactate Dehydrogenase}} \text{Lactate} + \text{NAD}$$

Reagents

Reagent	Description	Storage	Reagent Stability
REF 06860469	**ADVIA Chemistry Alanine Aminotransferase Concentrated (ALT_c) Reagents**		
Alanine Aminotransferase Concentrated Reagent 1 ALT_c R1	38 mL in 40-mL containers L-Alanine (1.22 mol/L) Lactate Dehydrogenase (LD, pig heart) (≥ 2.4 kU/L) NaN_3 (0.09%)	2–8°C	**Unopened:** Stable until the expiration date on product. **On-system:** 28 days
Alanine Aminotransferase Concentrated Reagent 2 ALT_c R2	27 mL in 40-mL containers α-Ketoglutarate (93 mmol/L) Reduced nicotinamide adenine dinucleotide (NADH) (1.41 mmol/L) NaN_3 (0.09%)	2–8°C	**Unopened:** Stable until the expiration date on product. **On-system:** 28 days
REF 10729782	**ADVIA Chemistry Pyridoxal-5'-Phosphate Liquid (P5P_L) Reagent**		
P5P_L	0.675 mL Pyridoxal-5'-Phosphate (30 mmol/L NaN_3 (0.09%)	2–8°C	**Unopened:** Stable until the expiration date on product. **On-system:** 28 days

Warnings and Precautions

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

Caution

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 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.

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.

Specimen Collection and Handling

Siemens Healthcare Diagnostics validated serum and plasma (lithium heparin) for the ADVIA Chemistry ALTPLc assay.

Follow these guidelines for specimens used for this assay:

- Serum and plasma can be collected using recommended procedures for collection of diagnostic blood specimens by venipuncture.[2] Follow the instructions provided with your specimen collection device for use and processing.[3]
- 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.[4]
- Specimens should be free of particulate matter.
- Specimens should be as fresh as possible.

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.

Procedure

Materials Provided

The following materials are provided:

Item	Contents	Number of Tests
REF 06860469	Reagent 1: 6 × 40-mL containers Reagent 2: 6 × 40-mL containers	6 × 820
REF 10729782	Pyridoxal-5'-Phosphate Liquid: 6 × 0.675 mL	6 × 820

Materials Required but Not Provided

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

Item	Description
REF 10916058	ADVIA Chemistry Enzyme 2 Calibrator
REF 10719152	40-mL reagent container adapter for 70-mL slot
	Commercially available control materials

Assay Procedure

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

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

Preparing the System

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

Preparing the Reagents

Use the following reagents to run the ADVIA Chemistry ALTPLc assay:

- ADVIA Chemistry Alanine Aminotransferase Concentrated (ALT_c) kit (REF 06860469)
- ADVIA Chemistry Pyridoxal-5'-Phosphate Liquid (P5P_L) reagent (REF 10729782)

The ADVIA Chemistry P5P_L reagent is supplied ready for use, it may be added directly into the ADVIA Chemistry ALT_c R1 wedge:

1. Carefully open one vial of the ADVIA Chemistry P5P_L and the ADVIA Chemistry ALT_c R1 wedge.
2. The P5P_L may be poured or pippetted into the ALT_c R1 wedge.
   - While holding the R1 wedge steady on the bench, carefully pour the contents of the P5P_L vial into the R1 wedge.

     Note 500 µL of P5P_L will be added to the R1 wedge

     Note Do not shake the excess P5P_L out of the vial.

     or
   - Using a volumetric pipette, withdraw 500 µL of P5P_L from the vial and carefully dispense it into the R1 wedge.
3. Carefuly close the R1 wedge
4. To ensure homogeneity, mix the contents by gently swirling the R1 wedge.

   Note Avoid formation of foam.

   Note Do not shake.

The R2 reagent is ready for 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.

ADVIA Chemistry systems automatically dilute ADVIA Chemistry concentrated reagents on a per-test basis.

Preparing the Samples

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 ALTPLc reagents are stable on the system for 28 days.

Do not use reagents beyond the expiration date.

Performing Calibration

To calibrate the ADVIA Chemistry ALTPLc assay, use the ADVIA Chemistry Enzyme 2 Calibrator (REF 10916058).

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 5 days.

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
- After replacing critical optical or hydraulic components
- 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
- When indicated by quality control procedures

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

Reagent Blank (RBL) Frequency

Run an RBL every day.

Note Use deionized water as the sample for the RBL in the ADVIA Chemistry ALTPLc 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 alanine aminotransferase 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 system automatically calculates and reports results based on the absorbance measurements of the test sample during the test, and of the calibrator(s) from calibration.

The instrument calculates the concentration of alanine aminotransferase in U/L (common units or SI units).

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.[5]

Sulfasalazine concentrations in serum or plasma above 50 mg/L may cause falsely depressed results with the ADVIA Chemistry ALTPLc assay.[6]

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.

Expected Values

The reference range for alanine aminotransferase is 7–40 U/L.[7] Siemens has verified the transference of reported reference range for the ADVIA Chemistry ALTPLc assay.[6]

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 9–1000 U/L for serum and plasma.

Results that are below the low end of the assay range are flagged **L**. You should report the test result as < 9 U/L.

Results that are above the high end of the assay range are flagged **H**.

Extended Measuring Range

Siemens has validated an automatic rerun condition for this assay that extends the reportable range to 6000 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) ≤ 8 U/L
- A limit of detection (LoD) ≤ 8 U/L
- A limit of quantitation (LoQ) ≤ 16 U/L

The LoB, LoD, and LoQ were determined as described in CLSI document EP17-A2.[8]

The LoB is the highest measurement result that is likely to be observed on a blank sample. The LoB for the ADVIA Chemistry ALTPLc assay is 2 U/L.

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 ALTPLc assay is 4 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 450 determinations with 225 blank and 225 low-level sample replicates.

The LoQ is the lowest amount of alanine aminotransferase that can be determined quantitatively within a defined total error. The LoQ for the ADVIA Chemistry ALTPLc assay is 9 U/L.

Precision

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

- ≤ 5.0% at 30–100 U/L
- ≤ 4.0% at 101–1000 U/L

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

- ≤ 7.0% at 30–100 U/L
- ≤ 5.0% at 101–1000 U/L

Each sample was assayed 2 times per run, 2 runs per day, for 20 days. The precision of the assay was analyzed as described in CLSI document EP05-A2.[9]

ADVIA Chemistry 1800 system

			Repeatability (Within-Run)		Within-Lab (Total)	
Specimen Type	N	Mean (U/L)	SD[a] (U/L)	CV[b] (%)	SD[a] (U/L)	CV[b] (%)
Serum Control 1	80	35.9	0.9	2.4	1.8	4.9
Serum Control 2	80	88.6	1.1	1.2	3.2	3.6
Serum Control 3	80	203.6	1.3	0.6	6.3	3.1
Serum Pool 1	80	544.4	3.1	0.6	12.1	2.2
Serum Pool 2	80	873.7	5.0	0.6	22.4	2.6
Plasma Pool	80	52.3	1.9	3.6	2.3	4.3

[a] SD = standard deviation
[b] CV = coefficient of variation

ADVIA Chemistry 2400 system

			Repeatability (Within-Run)		Within-Lab (Total)	
Specimen Type	N	Mean (U/L)	SD[a] (U/L)	CV[b] (%)	SD[a] (U/L)	CV[b] (%)
Serum Control 1	80	34.5	0.8	2.2	1.2	3.5
Serum Control 2	80	88.2	1.0	1.1	2.6	3.0
Serum Control 3	80	205.3	1.4	0.7	4.9	2.4
Serum Pool 1	80	550.7	4.4	0.8	9.2	1.7
Serum Pool 2	80	867.5	8.3	1.0	33.5	3.9
Plasma Pool	80	51.4	1.4	2.7	1.9	3.7

[a] SD = standard deviation
[b] CV = coefficient of variation

Actual results will vary depending on the study design, and on the sample and sample population used. Results obtained at individual laboratories may vary from the data provided.

Accuracy / Method Comparison

The ADVIA Chemistry ALTPLc assay is designed to recover analyte samples across the assay range with a slope of 1 ± 0.1 U/L when compared to the IFCC standard or the predicate ADVIA Chemistry ALTP_c assay. The performance of the ADVIA Chemistry ALTPLc assay (y) was compared with the performance of the comparison assay on the indicated system (x) as described in CLSI document EP09-A3.[10]

Specimen Type	Comparison Assay (x)	N	r	Regression Equation	Sample Range
Serum	IFCC Reference Assay	101	1.00	y = 0.96x - 1.6 U/L	12–1016 U/L
Serum	ADVIA Chemistry ALTP_c assay on the ADVIA Chemistry 1800 system	104	1.00	y = 1.03x + 0.2 U/L	10–950 U/L

The performance of the ADVIA Chemistry ALTPLc assay on the ADVIA Chemistry 1800 system (x) was compared with the performance of the assay on the indicated system (y).

Specimen Type	Comparison System (y)	N	r	Regression Equation	Sample Range
Serum	ADVIA Chemistry ALTPLc assay on the ADVIA Chemistry 2400 system	104	1.00	y = 1.00x - 0.4 U/L	11–976 U/L
Serum	ADVIA Chemistry ALTPLc assay on the ADVIA Chemistry XPT system	104	1.00	y = 1.01x - 0.1 U/L	11–976 U/L

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.

Matrix Comparison

To confirm the equivalency of sample types, the performance of the ADVIA Chemistry ALTPLc assay was compared for serum and lithium heparin plasma samples. Testing was performed using one lot of reagents and a single replicate from a matched set of serum and plasma samples in accordance with CLSI document EP09-A3.[10]

ADVIA Chemistry 1800 System

Specimen Type (x)	Comparison Specimen (y)	N	Regression Equation	Sample Range
Serum	Plasma (lithium heparin)	50	y = 0.99x - 0.3 U/L	15–916 U/L

ADVIA Chemistry 2400 System

Specimen Type (x)	Comparison Specimen (y)	N	Regression Equation	Sample Range
Serum	Plasma (lithium heparin)	50	y = 0.98x - 0.5 U/L	13–911 U/L

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

Interferences

Siemens tested the following potential interferents and found the results shown below.[11]

Note Turbidity resulting from equivalent levels of Intralipid and naturally occurring triglycerides in actual patient samples may not correlate.[12]

ADVIA Chemistry 1800 system

Interferent	Interferent Level	Alanine Aminotransferase Sample Concentration	Interference
Bilirubin (conjugated)	20 mg/dL	64 U/L	NSI[a]
	20 mg/dL	294 U/L	NSI
Bilirubin (unconjugated)	20 mg/dL	64 U/L	NSI
	20 mg/dL	293 U/L	NSI
Lipemia (triglycerides from Intralipid)	400 mg/dL	60 U/L	NSI
	400 mg/dL	272 U/L	NSI

Interferent	Interferent Level	Alanine Aminotransferase Sample Concentration	Interference
Hemolysis (hemoglobin)	200 mg/dL	61 U/L	NSI
	200 mg/dL	283 U/L	NSI
Sulfasalazine[b]	50 mg/L	65 U/L	NSI
	50 mg/L	297 U/L	NSI
Sulfapyridine	299 mg/L	62 U/L	NSI
	299 mg/L	283 U/L	NSI

[a] NSI = No significant interference. A percentage effect > 10% is considered a significant interference.
[b] Refer to Limitations.

ADVIA Chemistry 2400 system

Interferent	Interferent Level	Alanine Aminotransferase Sample Concentration	Interference
Bilirubin (conjugated)	20 mg/dL	66 U/L	NSI[a]
	20 mg/dL	296 U/L	NSI
Bilirubin (unconjugated)	20 mg/dL	66 U/L	NSI
	20 mg/dL	297 U/L	NSI
Lipemia (triglycerides from Intralipid)	400 mg/dL	64 U/L	NSI
	400 mg/dL	293 U/L	NSI
Hemolysis (hemoglobin)	200 mg/dL	62 U/L	NSI
	200 mg/dL	287 U/L	NSI
Sulfasalazine[b]	50 mg/L	66 U/L	NSI
	50 mg/L	299 U/L	NSI
Sulfapyridine	299 mg/L	66 U/L	NSI
	299 mg/L	287 U/L	NSI

[a] NSI = No significant interference. A percentage effect > 10% is considered a significant interference.
[b] Refer to Limitations.

Actual results will vary depending on the study design, the levels of the potential interferences tested, and the samples used. Results obtained at individual laboratories may vary from the data provided.

Standardization

The ADVIA Chemistry ALTPLc assay is traceable to the IFCC reference method via patient sample correlation.[13] Refer to the correlation data in the *Accuracy/Method Comparison* section for the relationship.

Technical Assistance

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

siemens.com/healthcare

References

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12. Twomey PJ, Don-Wauchope AC, McCullough D. Unreliability of triglyceride measurement to predict turbidity induced interference. *J Clin Pathol.* 2003 Nov;56(11):861–862.
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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

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