Pointe Alkaline Phosphatase Reagent Set

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853

Intended Use
For the quantitative determination of alkaline phosphatase in human serum using the Yumizen C560 analyzer. **Rx Only.**

Clinical Significance
Serum alkaline phosphatase estimations are of interest in the diagnosis of two groups of conditions; hepatobiliary disease and bone disease associated with increased osteoblastic activity.[1]

Test Summary
Alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of various phosphate esters under specified conditions. ρ-Nitrophenyl Phosphate is one such phosphate ester and was introduced as a substrate by Fujita in 1939.[2]
Bessey, Lowry, and Brock published an endpoint procedure in 1946[3] while Bowers and McComb reported a kinetic procedure in 1966.[4] The kinetic procedure has undergone several modifications and been recommended for routine analysis.[5,6] This liquid reagent is based on the recommended method of the AACC.[7]

Principle
$$\rho\text{-NPP} + H_2O \xrightarrow{\text{Alk. Phos.}} \rho\text{-Nitrophenol} + H_3PO_4$$
ρ-Nitrophenyl phosphate is hydrolyzed to ρ-nitrophenol and inorganic phosphate. The rate at which the p-NPP is hydrolyzed, measured at 405 nm, is directly proportional to the alkaline phosphatase activity.

Reagent Composition
After combining R1 and R2 as directed the reagent contains: AMP Buffer (pH 10.45), ρ-NPP ≤16mM, Magnesium ions ≥1.0mM, activators and preservatives.

Reagent Preparation
The reagents are ready to use.

Reagent Storage and Stability
Store reagent set at 2-8°C. The reagents are stable until the expiration date if stored as directed. Protect from direct light and avoid microbial contamination. **NOTE:** The R2 reagent is temperature sensitive and can be affected by prolonged exposure to room temperature. Return reagent to 2-8°C as soon as possible after use. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

Precautions and Hazards
1. This reagent set is for *in vitro* diagnostic use only. Do not ingest any material.
2. Do not use if the initial absorbance of the working reagent is greater than 1.0 at 405 nm or if the reagent fails to meet the stated parameters of performance.
3. Reagent should not be used if it fails to recover stated values in control sera or shows evidence of microbial contamination.
4. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Ed., 1988, HHS Publication No. (CDC) 88-8395.

Hazards:
R1: Hazard Classifications: Skin Corrosion/Irritation (Category 1), Eye Damage/Irritation (Category 2), Respiratory Sensitizer (Category 1), Specific Target Organ Toxicity, Single Exposure; Respiratory System (Category 1), Specific Target Organ Toxicity, Repeat Exposure; Respiratory System and Teeth (Category 1)
Hazard Statements: H314: Causes severe skin burns and eye damage, H319: Causes serious eye irritation, H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled, H370: Causes damage to organs, H372: Causes damage to organs through prolonged or repeated exposure.
Precautionary Statements: **Prevention:** P260: Do not breathe dust/fume/gas/mist/vapor/spray, P264: Wash skin thoroughly after handling, P270: Do not eat, drink or smoke when using this product, P280: Wear protective gloves/protective clothing/eye protection/face protection, P285: In case of inadequate ventilation wear respiratory protection, **Response:** P310: Immediately call a POISON CENTER or doctor/physician, P314: Get medical advice/attention if you feel unwell, P363: Wash contaminated clothing before reuse. P301 + P330 + P331: IF SWALLOWED: rinse mouth. Do NOT induce vomiting. P303 + P361 + P353: IF ON SKIN (or hair): Remove/Take off Immediately all contaminated clothing. Rinse SKIN with water/shower, P304 + P340: IF INHALED: Remove victim to fresh air and Keep at rest in a position comfortable for breathing, P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. P337 + P313: IF eye irritation persists: Get medical advice/attention. **Storage:** P404: Store in a closed container. **Disposal:** P501: Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations.


Signal Word: Danger

R2: Hazard Classifications: Skin Corrosion/Irritation (Category 2), Eye Damage/Irritation (Category 2), Specific Target Organ Toxicity, Single Exposure; Liver, Kidney, Central Nervous System, Eyes (Category 2), Specific Target Organ Toxicity, Repeated Exposure; Liver, Kidney, Central Nervous System (Category 2), Reproductive Toxicity (Category 2), Germ Cell Mutagenicity (Category 2), Carcinogenicity (Category 2)
Hazard Statements: H315: Causes skin irritation, H319: Causes serious eye irritation, H341: Suspected of causing genetic defects, H351: Suspected of causing cancer, H361: Suspected of damaging fertility or the unborn child, H371: May cause damage to organs, H373: Causes damage to organs through prolonged or repeated exposure
Precautionary Statements: **Prevention:** P202: Do not handle until all safety precautions have been read and understood, P260: Do not breathe dust/fume/gas/mist/vapors/spray, P264: Wash skin thoroughly after handling, P270: Do not eat, drink or smoke when using this product, P280: Wear protective gloves/protective clothing/eye protection/face protection.
Response: P314: Get medical advice/attention if you feel unwell, P362: Take off contaminated clothing and wash before use, P302 + P352: IF ON SKIN: wash with plenty of soap and water. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. P309 + P311: IF exposed or if you feel unwell: call a POISON CENTER or doctor/physician, P332 + P313: IF SKIN irritation occurs: Get medical advice/attention, P337 + P313: IF eye irritation persists: Get medical advice/attention. **Storage:** P404: Store in a closed container. **Disposal:** P501: Dispose of contents to an approved waste disposal plant. **Refer to the Safety Data Sheet for this product (SDS-A7516) available by calling 1-734-487-8300.**

Specimen Collection and Storage
1. Use non-hemolyzed serum (plasma should not be used since anticoagulant agents inhibit alkaline phosphatase activity).[8,9]
2. Serum samples should be stored at 2-8°C and run within two days.[10]
3. Specimen collection should be carried out in accordance with NCCLS M29-T2.[11] No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Interferences

1. Young, et al[8] provide a list of drugs and other substances that interfere with the determination of ALP activity.

Materials Provided

Alkaline Phosphatase, catalog number: 14-A7516-225

Materials Required but not Provided

1. Yumizen C560 Analyzer.
2. Yumizen C560 Operation manual.
3. Chemistry Control, catalog number C7592-100

Limitations

1. This methodology measures total alkaline phosphatase irrespective of tissue or organ of origin. Further tests may be necessary to assist in differential diagnosis.
2. Samples with values exceeding 1000 IU/L should be diluted with an equal volume of saline and re-assayed multiplying the results by two.

Calibration

The procedure is standardized by means of the millimolar absorptivity of ρ-nitrophenol (18.75 at 405nm) under the specified conditions. Results are based on the change in absorbance per unit of time; all parameters must be known and controlled.

Quality Control

The validity of the reaction should be monitored using control sera with known normal and abnormal ALP activities and should be run with every working shift in which ALP assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values

Adults 35-123 IU/L at 37°C. This reference range is based on a study performed by the manufacturer using samples from 783 apparently healthy adults. Children have a higher normal value. It is strongly suggested that each laboratory establish its own normal range.

Performance

1. Assay Range: 2-1000 IU/L
2. Comparison: A study was performed between the Yumizen C560 and a similar analyzer and method, resulted in the following:

Method	Alkaline Phosphatase
N	81
Mean Alk. Phos. (IU/L)	126.5
Range (IU/L)	19-833
Standard Deviation	147.7
Regression Analysis	y = 1.063x – 9.7
Correlation Coefficient	0.9987

3. Precision: Precision studies were performed using the Yumizen C560 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[13]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	88.6	302.1	887.3
Standard Deviation	1.1	1.2	3.6
Coefficient of Variation (%)	1.3%	0.4%	0.4%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	89.5	311.2	870.7
Standard Deviation	4.2	12.5	23.0
Coefficient of Variation (%)	4.7%	4.0%	2.6%

4. Sensitivity: 2SD Limit of Detection (95% Conf) = 2 IU/L

References

1. Tietz, N.W., Fundamentals of Clinical Chemistry, W.B. Saunders co., p 603 (1982).
2. Fujita, H., J. Biochem, (Japan) 30:69 (1969).
3. Bessey, O.A., Lowry, O.H., Brock, M.J., J. Biol. Chem. 164:321 (1964).
4. Bowers, G.N., Jr., McComb, R.B., Clin. Chem. 12:70 (1966).
5. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, Scand. J. Clin. Lab. Invest 32:291 (1974).
6. Wilkinson, J.H., et al, Clin. Chem. 15:487 (1969).
7. Tietz, N.W., et al, Clin. Chem. 29:751 (1983).
8. Young, D.S., et al, Clin. Chem. 21:1D (1975).
9. Demetriou, J.A., Drewes, P.A., Gin, J.B., Clinical Chemistry: Principles and Technics, 2nd Ed., Hagerstown (MD), Harper & Row, p. 927 (1974).
10. Rej., R., Clin. Chem. 23:1903 (1977).
11. NCCLS document "Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue", 2nd Ed. (1991).
12. NCCLS document "Interference testing in Clinical Chemistry", 2nd Ed. (1992).
13. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

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CHEMISTRY PARAMETERS

Chem:	ALKP No.: 201	Sample Type:	Serum
Chemistry:	Alkaline Phosphatase	Print Name:	ALKP
Reaction Type:	Kinetic	Reaction Direction:	Positive
Pri Wave:	412	Sec Wave:	660
Unit:	U/L	Decimal	0
Blank Time:	0 0	Reaction Time:	56 71

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	3.0 ul	--- ul	--- ul	R1: 120 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	2	1000	Linearity Limit:	0.3
Linearity Range (Decreased)	____	____	Substrate Depletion:	25000
Linearity Range (Increased)	____	____	Mixed Blank Abs:	
R1 Blank Abs:	____	____	Uncapping Time	
Blank Response:	____	____	Reagent Alarm Limit:	
Twin Chemistry:			☐ Enzyme Linear Extension	

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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CALIBRATION PARAMETERS

Calibrator Definition

Calibrator:	*	Lot No.:	*
Exp Date:	*		

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	ALKP	0	U/L

Calibration Setup

Chem: ALKP

Calibration Settings

Math Model:	K Factor		
Factor:	2708	Replicates:	1

Acceptance Limits

Cal Time:	24	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF 14-A7516-225

Manufactured by
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2°C – 8°C IVD

Certified to Perform Reagents
The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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CE

Symbol Key

Use by (YYYY-MM-DD) LOT Lot and batch code REF Catalog number
Manufacturer Temperature limitation Consult instructions for use
IVD *In vitro* diagnostic medical device **Rx Only:** Prescription Use Only