ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

DetectX®

Alkaline Phosphatase Colorimetric Activity Kit

2 Plate Kit Catalog Number K082-H1

Species Independent

Sample Types Validated:

Serum, non-EDTA plasma, and other biological samples

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

WEB INSERT 220127

TABLE OF CONTENTS

BACKGROUND

Alkaline phosphatase (ALP; - E.C.3.I.3.1.) catalyzes the alkaline hydrolysis of phosphate esters on a large variety of naturally occurring and synthetic substrates, producing an organic radical and inorganic phosphate.[1,3,5] ALP is found in many organisms, both prokaryotic and eukaryotic. The enzyme plays an active role in regulating many biological processes in higher organisms, ranging from metabolism, signal transduction, molecule transportation, and the expression of genetic information.[2,6]

In humans there are several forms depending upon the site of tissue expression; Intestinal ALP, Placental ALP, Germ cell ALP and tissue nonspecific alkaline phosphatase or liver/bone/kidney (L/B/K) ALP.[2]

Each subunit of the homodimeric enzyme contains two Zn2+ ions and one Mg2+ ion, which occupy the active sites of the enzyme and are necessary cofactors for ALP activity. Maintaining this ratio of zinc and magnesium is crucial, and it is for this reason that anticoagulants such as citrate, oxalate, and EDTA must be avoided in samples, as they can bind these cations.[1,3,5]

The levels of ALP measured in the blood depends on factors such as age, sex, or blood type.[4] Blood levels of alkaline phosphatase also increase by two to four times during pregnancy. This is a result of additional alkaline phosphatase produced by the placenta.[7] Changes in alkaline phosphatase can also be a marker for several disease states mostly involving the skeletal system and liver.[1] Increases in ALP are observed in all forms of cholestasis, particularly with obstructive jaundice. It is also elevated in diseases of the skeletal system, such as Paget disease, hyperparathyroidism, rickets and osteomalacia, as well as with fractures and malignant tumors.[4,6]

1. PhD, R. N. (2017). Chapter 29. Serum Enzymes, Alkaline Phosphatase. In Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6e (6th ed., p. 415). Saunders.
2. Sharma, U., Pal, D., & Prasad, R. (2013). Alkaline Phosphatase: An Overview. Indian Journal of Clinical Biochemistry, 29(3), 269–278. https://doi.org/10.1007/s12291-013-0408-y
3. Coleman, J. E. (1992). Structure and Mechanism of Alkaline Phosphatase. Annual Review of Biophysics and Biomolecular Structure, 21(1), 441–483. https://doi.org/10.1146/annurev.bb.21.060192.002301
4. Mayo Clinic Laboratories. (2021, September). Test: Alkaline Phosphatase, Serum. https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/8340
5. Kim, E. E., & Wyckoff, H. W. (1991). Reaction mechanism of alkaline phosphatase based on crystal structures. Journal of Molecular Biology, 218(2), 449–464. https://doi.org/10.1016/0022-2836(91)90724-k
6. Tang, Z., Chen, H., He, H., & Ma, C. (2019). Assays for alkaline phosphatase activity: Progress and prospects. Trends in Analytical Chemistry, 113, 32–43. https://doi.org/10.1016/j.trac.2019.01.019
7. Shipman, K. E., Holt, A. D., & Gama, R. (2013). Interpreting an isolated raised serum alkaline phosphatase level in an asymptomatic patient. BMJ, 346(apr03 2), 1–4. https://doi.org/10.1136/bmj.f976

ASSAY PRINCIPLE

The DetectX® Alkaline Phosphatase Colorimetric Activity Kit is an exclusively licensed assay kit designed to quantitatively measure alkaline phosphatase (ALP) activity in a variety of samples. Please read the complete kit insert before performing this assay. A calibrated bovine ALP standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Samples are diluted in the provided Assay Buffer and added to the wells of a clear half area well plate. Substrate is then added to each well and the plate incubated at 37°C for 30 minutes. The ALP reacts with the substrate to convert the colorless substrate into a yellow-colored product. The colored product is read at 405 nm. Increasing levels of ALP in the samples directly correlate with an increase in yellow product. The activity of the ALP in the sample is calculated after making a suitable correction for dilution, using software available with most plate readers. The results are expressed in terms of milliunits of ALP activity per mL, or mU/mL.

Assay Kit developed by 21 Grams Assays, Inc., www.21gramsassays.com.

RELATED PRODUCTS

Kits	Catalog No.
Arg^8-Vasopressin (AVP) Chemiluminescent ELISA Kits	K049-C1/C5
Arg^8-Vasopressin ELISA Kits	K049-H1/H5
C-Reactive Protein (CRP) Human ELISA Kits	K069-H1/H5
Creatinine Serum Detection Kits	KB02-H1/H2
Galactose Colorimetric Detection Kit	K042-H1
Myeloperoxidase (MPO) Human ELISA Kit	K060-H1
PGE_2 Multi-Format ELISA Kits (Strip Wells and Whole Plate)	K051-H1/H5/H1W/H5W
Retinol Binding Protein (RBP) Multi-Format ELISA Kits	K062-H1/H5
ST2 Human ELISA Kit	K055-H1

SUPPLIED COMPONENTS

Clear Half Area 96 Well Plates
Corning CoStar Plate 3695

2 Plates — Catalog Number X018-2EA

Alkaline Phosphatase Standard
A calibrated standard containing 10,000 mU/mL of bovine alkaline phosphatase in a special solution.

90 µL — Catalog Number C294-90UL

Assay Buffer Concentrate
A 5X buffer concentrate containing detergents and stabilizers that must be diluted in dionized or distilled water.

25 mL — Catalog Number X155-25ML

pNPP Substrate
A solution of colorimetric substrate in a special stabilizing buffer.

5 mL — Catalog Number X156-11ML

Stop Solution
A 0.1M solution of trisodium phosphate.

6 mL — Catalog Number X158-6ML

Plate Sealer
2 each — Catalog Number X002-1EA

STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Repeater pipet with disposable tips capable of dispensing 50 µL and 25 µL.

Incubator capable of maintaining 37˚C.

96 well plate reader capable of reading optical density at 405 nm (Acceptable Range 405-410 nm).

Software for converting fluorescent intensity readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

SAMPLE TYPES

This assay has been validated for serum and plasma containing non-chelating anticoagulant. Samples containing visible particulate should be centrifuged prior to using. Avoid samples that are hemolyzed and those containing chelators like citrate, oxolate, and EDTA. The end user should evaluate recoveries in other samples being tested.

SAMPLE PREPARATION

Serum

1. Collect serum in tubes without anticoagulant. Allow to clot for 30 minutes at room temperature. Centrifuge the sample at 2,000 x g for 15 minutes at 4°C. Aspirate off the pale yellow serum without disturbing the white buffy layer.

2. Assay immediately or freeze at ≤ -70°C in aliquots for later use.

3. Serum must be diluted ≥ 1:4 by taking one part of serum and adding 3 parts of diluted Assay Buffer prior to assaying. Further dilutions in Assay Buffer may be needed.

Plasma

1. Collect plasma in tubes with non-chelating anticoagulant. Centrifuge at 700-1,000 x g for 10 minutes at 4°C. Aspirate off the pale yellow plasma without disturbing the white buffy layer.

2. Assay immediately or freeze at ≤ -70˚C in aliquots for later use.

3. Plasma must be diluted ≥ 1:4 by taking one part of plasma and adding 3 parts of diluted Assay Buffer prior to assaying. Further dilutions in Assay Buffer may be needed.

Use all samples within 2 hours of dilution.

STANDARD PREPARATION

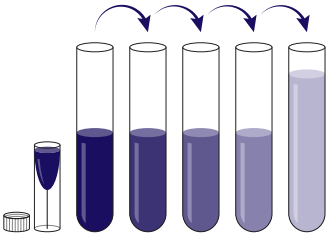
Allow the kit reagents to come to room temperature for 30-60 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Standard Preparation

Standards are prepared by labeling tubes as #1 through #7. Add 495 µL of Assay Buffer to tube #1. Pipet 100 µL of Assay Buffer into tubes #2 to #7. Carefully add 5 µL of the Alkaline Phosphatase Stock from the vial to tube #1 and vortex completely. Take 100 µL of the alkaline phosphatase solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The alkaline phosphatase activity in tubes 1 through 7 will be 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 mU/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Vol (µL)	495	100	100	100	100	100	100
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	5	100	100	100	100	100	100
Final Activity (mU/mL)	100	50	25	12.5	6.25	3.125	1.563

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Alkaline Phosphatase activities.

Use the plate layout sheet on the back page to aid in proper sample and standard identification. Set plate parameters for a 96-well Corning Costar 3695 plate. See: www.ArborAssays.com/resources/#general-info for plate dimension data.

1. Pipet 10 µL of samples or appropriate standards into duplicate wells in the plate.
2. Pipet 10 µL of diluted Assay Buffer into duplicate wells as the Zero standard.
3. Add 50 µL of the supplied Substrate to each well using a repeater pipet.
4. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer.
5. Incubate the plate at 37˚C for 30 minutes without shaking.
6. Add 25 µL of the Stop Solution to each well using a repeater pipet.
7. Read the optical density from each well in a plate reader capable of reading at 405 nm (acceptable Range 405 - 410 nm).
8. Use the plate reader's built-in 4PLC software capabilities to calculate alkaline phosphatase activity in each sample.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader. The sample activity obtained should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:

https://www.myassays.com/

TYPICAL DATA

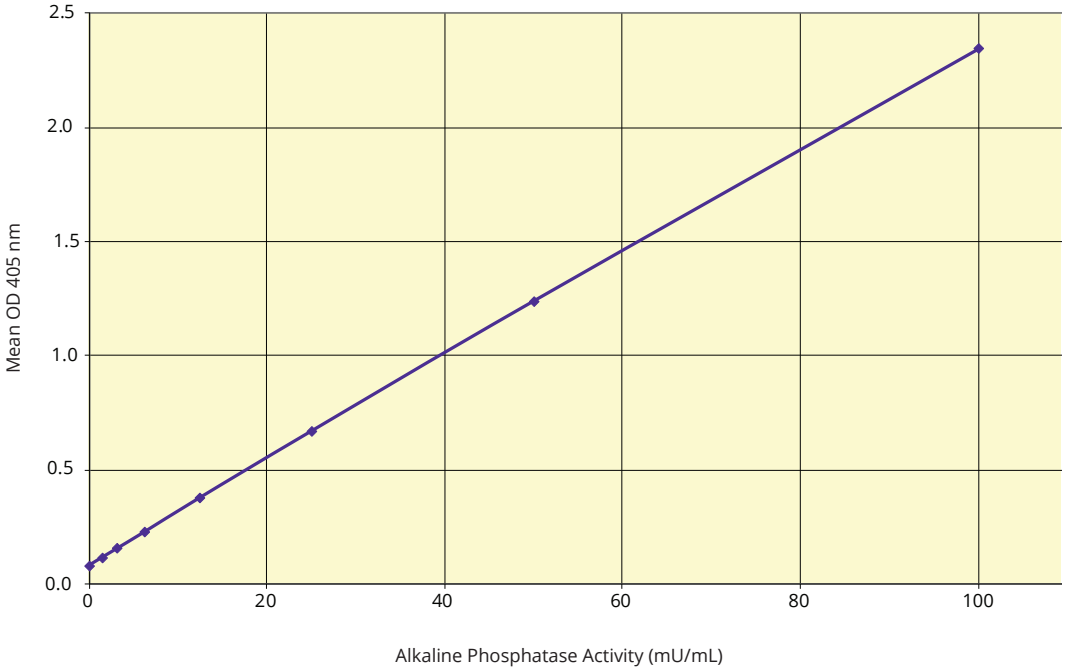
Sample	Mean OD	ALP Activity (mU/mL)
Standard 1	2.342	100
Standard 2	1.238	50
Standard 3	0.668	25
Standard 4	0.377	12.5
Standard 5	0.226	6.25
Standard 6	0.153	3.125
Standard 7	0.116	1.563
Zero	0.079	0
Sample 1	1.049	41.68
Sample 2	0.455	16.06

Always run your own standard curves for calculation of results. Do not use this data.

Alkaline Phosphatase Unit Definition

One unit will hydrolyze 1.0 micromole of p-nitrophenyl phosphate to p-nitrophenol and inorganic phosphate per minute at 37˚C and pH 9.8.

Typical Standard Curve



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

Sensitivity was determined as 0.063 mU/mL.

Limit of Detection

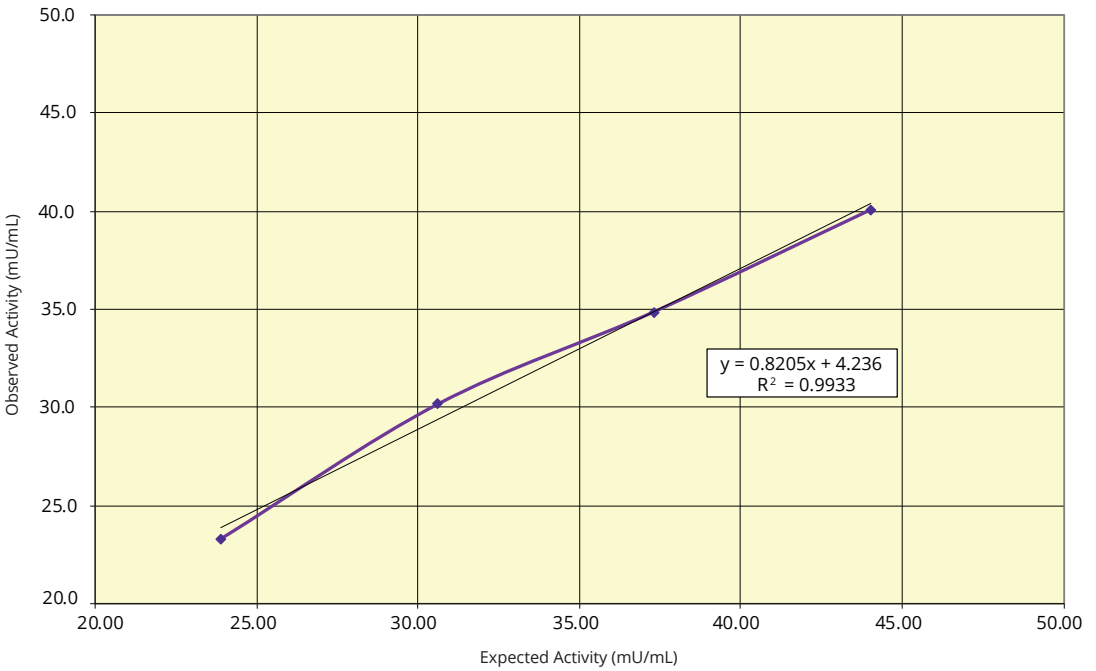
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample.

Limit of Detection was determined as 0.060 mU/mL.

Linearity

Linearity was determined by taking two serum samples, one with a high known alkaline phosphatase activity of 50.73 mU/mL and the other with a lower alkaline phosphatase activity of 17.22 mU/mL, and mixing them in the ratios given below. The measured activities were compared to the expected values based on the ratios used.

High Sample	Low Sample	Expected Activity (mU/mL)	Observed Activity (mU/mL)	% Recovery
80%	20%	44.03	40.08	91.0
60%	40%	37.33	34.88	93.4
40%	60%	30.62	30.17	98.5
20%	80%	23.92	23.32	97.5
			Mean Recovery	**95.1%**



Intra Assay Precision

Three samples diluted in Assay Buffer were run in replicates of twenty in an assay. The mean and precision of the calculated activities were:

Sample	ALP Activity (mU/mL)	%CV
1	70.46	1.6
2	40.45	1.1
3	15.54	1.4

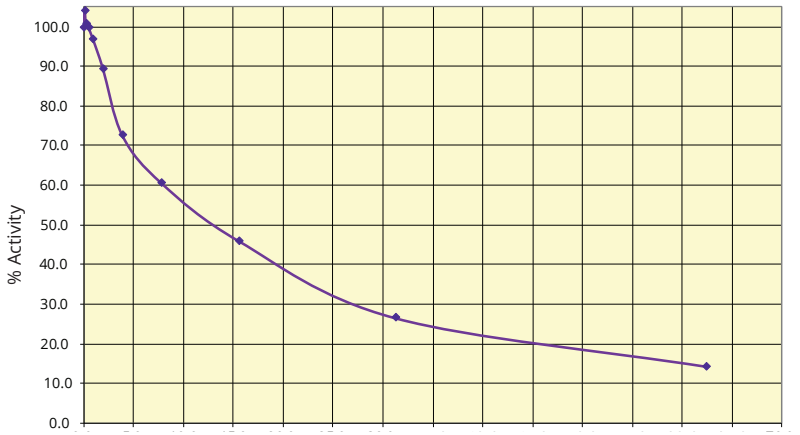
Inter Assay Precision

Three samples diluted in Assay Buffer were run in duplicates in twenty assays run over multiple days by multiple operators. The mean and precision of the calculated activities were:

Sample	ALP Activity (mU/mL)	%CV
1	72.86	6.4
2	41.68	8.1
3	16.06	9.4

Inhibition Studies

The bovine ALP standard was incubated with varying concentrations of a reversible inhibitor of ALP activity, Levamisole, from 0.12 to 62.5 mM for 2 hours at room temperature in the kit Assay Buffer. The activity in the incubated samples was then determined in the assay.



Interferents

A variety of organic solvents were tested as possible interfering substances in the assay by comparing their activity to those generated by an Assay Buffer control sample.

Addition	%Maximum Dose	% Change
DMSO	1	4.21
DMF	1	9.79
MeOH	10	1.36
EtOH	5	4.66

SAMPLE VALUES

Seventeen normal adult human serum samples and six normal adult human heparin plasma samples were diluted in Assay Buffer between 1:4 and 1:64 and run in the assay. Additionally, nine serum samples from select disease states, shown below, were diluted 1:8 in Assay Buffer and run in the assay. Normal serum samples ranged from 40 – 80 mU/mL with an average of 60.5 mU/mL after adjusting for dilution. Normal heparin plasma samples ranged from 35.9 – 80 mU/mL with an average of 52.4 mU/mL after adjusting for dilution.

Sample	Disease State/Condition	ALP (mU/mL)
1	Male, 34, Nonalcoholic Steatohepatitis (NASH) liver disease	190.4
2	Female, 34, Nonalcoholic Steatohepatitis (NASH) liver disease	189.0
3	Male, 75, Liver cancer	267.8
4	Female, 75, Liver cancer	83.3
5	Male, 63, Osteoarthritis	233.4
6	Female, 49, Osteoarthritis	149.5
7	Male, 51, Osteoarthritis	192.9
8	Female, 31, pregnancy (15 weeks)	79.8
9	Female, 34, pregnancy (34 weeks)	141.9

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com

OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

H	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12