Brown Clinic Northridge
511 14th Ave. NE
Watertown, SD

Brown Clinic
506 First Ave. SE
Watertown, SD

BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Alkaline Phosphatase

PURPOSE:
The ALP method used on the Dimension EXL200 integrated chemistry system is an *in vitro* diagnostic test intended for the quantitative determination of alkaline phosphatase activity in **serum** and **plasma**. Measurements of alkaline phosphatase or its isoenzymes are used in the diagnosis and treatment of liver, bone, parathyroid and intestinal disorders.

PRINCIPLE:
The alkaline phosphatase method is based on a procedure published by Bowers and McComb[1] and more recently reviewed by Rej.[2] This method responds to all ALP isoenzymes in human serum.

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 405 nm due to the formation of p-NP is directly proportional to the ALP activity, since other reactants are present in non-rate limiting quantities and is measured using a bichromatic (405, 510 nm) rate technique.

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

INSTRUMENT, REAGENTS & SUPPLIES:

Reagents

Wells[a]	Form	Ingredient	Concentration[b]
1–6	Liquid	2-Amino-2Methyl-1-Propanol(AMP)	3.0 M
(Reagent 1)		Magnesium Acetate	8.0 mmol/L
		Zinc Sulfate	4.0 mmol/L
		HEDTA	8.0 mmol/L
7- 8	Liquid	p-NPP Buffer	101.6 mmol/L

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Nominal final value per well in a cartridge.

Risk and Safety:

Irritant. Contains 2-amino-2-methyl-1-propanol, Sulfuric Acid, Zinc, salt, Heptahydrate

R36/38: Irritating to eyes and skin

R52/53: Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety Data Sheets (SDS/MSDS) can be found at www.siemens.com/healthcare

Precautions:

Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact and ingestion.

For *in vitro* diagnostic use

Reagent Preparation: All reagents are liquid and ready to use.

STORAGE & STABILITY:
Store at 2 – 8° C
Protect from light after opening.

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed wells on the instrument are stable for 30 days.

Open Well Stability: 2days for wells 1-6
4 days for wells 7-8

SPECIMEN REQUIREMENTS:
Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.

- patient preparation: no patient preparation required
- specimen type: serum or heparinized plasma
- handling: stable for 8 hours at room temperature, 7 days at 2-8^{o}C, 6 months when frozen at -20^{o}C or colder.[6]
- Avoid repeated freezing and thawing, thawed specimens which are turbid must be clarified by centrifugation prior to testing.

CONTROLS:
At least once daily run solutions at two levels of a quality control material with known concentrations.

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

PROCEDURE:
ALPI Flex® reagent cartridge, Cat. No. DF150

Materials Required But Not Provided
ALPI CAL, Cat. No. DC150
Enzyme Diluent, Cat. No. 790035901
Quality Control Materials

Test Steps

Sampling[c], reagent delivery, mixing, processing and printing of results are automatically performed by the Dimension® system. For details of this processing, refer to your Dimension® system manual.

c. The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus the dead volume. Precise container filling is not required.

Test Conditions

- Sample Size: 7 µL
- Reagent 1 Volume: 90 µL
- Reagent 2 Volume: 57 µL
- Diluent Volume: 206 µL
- Test Temperature: 37° C
- Reaction Time: 7.2 minutes
- Wavelength: 405 and 510 nm
- Type of Measurement: Bichromatic rate

Backup:
Refer to Brown Clinic Backup Policy

Calibration:

Assay Range (@ 37° C): 10 – 1000 U/L[0.17-16.70 µkat/L][d]

Calibration Material: Dimension® ALPI CAL, Cat. No. DC150

Calibration Scheme:	Three levels (n=3)
Units:	U/L [μkat/L]
	(U/L x 0.0167)= [μkat/L]
Typical Calibration Levels:	Level 1: 0 U/L [0.00 μkat/L]
	Level 2: 500 U/L [8.35 μkat/L]
	Level 3: 1000 U/L [16.70 μkat/L]
Calibration Frequency:	Every 90 days for any one lot

A new calibration is required:

- For each new lot of Flex® reagent cartidges
- After major maintenance or service, if indicated by quality control results
- As indicated in laboratory quality control procedures
- When required by government regulations

Assigned Coefficients:	C0 0.000
	C_1 1.000

d. Systeme International d'Unites [SI Units] are in brackets.

INTERPRETATION:

The instrument automatically calculates and prints the activity of alkaline phosphatase in U/L using the calculation scheme illustrated in your Dimension® system manual.

Expected Values:

- 45-116 U/L

The reference interval was calculated non-parametrically and represents the central 95% of the population.

Each laboratory should establish its own reference interval for alkaline phosphatase as performed on the Dimension® system.

Reporting:

- report format: U/L

LIMITATIONS:

Results:	>1000 U/L
Manual dilution:	Results in excess of 1000 U/L should be repeated after diluting the sample with Enzyme Diluent (Cat. No. 790035901) or equivalent to produce a sample within the assay range.g Enter dilution factor. Reassay. Resulting readout is corrected for dilution.
Autodilution (AD):	Refer to your Dimension® system literature.

- Samples with results in excess of 1000 U/L[16.70 μkat/L]are reported as "Above Assay Rang" and should be repeated on dilution.
- Samples with results less than 10 U/L[0.17 μkat/L] will be reported as "less than 10U/L"

•A system malfunction may exist if the following 5-test precision is observed:

Activity	SD
150 U/L [2.51 μkat/L]	>7 U/L [0.12 μkat/L]
500 U/L [8.35 μkat/L]	>16 U/L [0.27 μkat/L]

Analytical Specificity

For Known Interfering Substances section refer to package insert.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert.

Reference: ALPI Flex® reagent cartridge insert sheet PN 717015.002 Issue Date 2015-01-30

Origination Date: 9-10-07
Date of Implementation: 11-10-10
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Written By: ____Lori Murray MT(ASCP)_____ Date: _8-8-12

Approved By: _____Aaron Shives, MD_____ Date: ___10/21/2017_____
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
07/10	Lori Murray	New format
8-8-12	Lori Murray	chg Instrumentation to EXL200
5-8-15	Sam Legg	updated information to package insert dated 09-04-2012
8-7-15	Heather Hall	Updated risk and safety
7/11/17	LMurray	ref range/ formatting
10/17/17	Heather Hall	Modified Backup Method