MA-0109

Alkaline Phosphatase Activity Assay

(100 wells, Colorimetric, OD 405 nm, Store at -20°C)

Background Information:

Alkaline phosphatase catalyzes the hydrolysis of phosphate esters under alkaline conditions with the production of free phosphate. While the physiological purpose of alkaline phosphatase is still a question, empirically, changes in the level of this enzyme are used in the diagnosis of a variety of disease states such as inflammation related to CKD and IBD, certain anemias and cancer. Akrivisbio's Alkaline Phosphatase Activity Assay, based on the hydrolysis of p-nitrophenol phosphate, is a simple, sensitive assay able to measure alkaline phosphatase activity in a variety of biological samples. There are two conventions used to describe Alkaline phosphatase activity, Glycine units and DEA units. We use Glycine units in our assay.

Assay Principle:

1 – p-nitrophenol phosphate is hydrolyzed to phosphate ion and p-nitrophenol
2 - p-nitrophenol formation is monitored at λmax = 405 nm)

Assay Components:

Assay Buffer	100 ml	NM	MA-0109A
pNPP	10 tablets	Red	MA-0109B
Alkaline Phosphatase	2 Units	White	MA-0109C
Stop Solution	10 ml	WM	MA-0109D
p-Nitrophenol Standard	500 µl	Yellow	MA-0109E

Storage and Handling: Store the kit at 4°C. Bring buffer to room temperature before using. Do not touch pNPP tablets with bare hands.
pNPP: Dissolve 1 tablet pNPP into 2.5 ml Assay Buffer, sufficient for 50 wells. Keep pNPP solution on ice. Use or discard same day, do not store overnight.
Alkaline phosphatase: Reconstitute with 100 µl of DI water. The enzyme is stable for up to 2 months at 4°C.

Assay Protocol:

1. Standard Curve Preparation: Add 0 – 5 – 10 – 15 – 20 - 25 µl of the 1.0 mM pNP standard into a series of wells in a 96-well plate in duplicate to generate 0 – 5 – 10 – 15 – 20 - 25 nmol/well pNP Standard. Bring the final volume to 100 µl with Assay Buffer.

2. Sample Preparation: A number of chemicals act as inhibitors of Alkaline phosphatase, such as EDTA, oxalate, fluoride, and citrate. These must be avoided, if at all possible, in samples. Dilute serum or plasma 10X. Cells (~10^5) are washed, pelleted and placed in 50 µl of Assay Buffer, then homogenized and centrifuged at 16,000 X g for 2-3 min. to remove insoluble materials. Transfer the clear supernatant to a fresh tube. Use 5-25 µl as a sample. Bring each well to 50 µl with Assay Buffer. Use two different amounts of each sample. Activity measured should be proportional to the amount of sample used for a measurement. If it's not, reduce or dilute the amount of sample used.

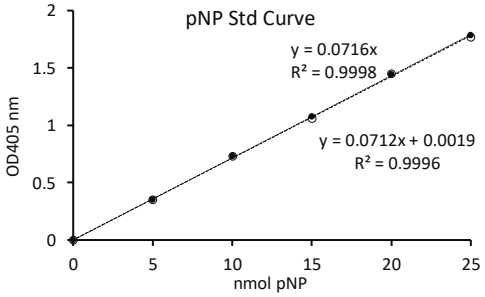
3. Positive Control: Dilute the Positive Control 20X by adding 2 µl to 48 µl Assay Buffer. Final concentration is ~ 1 mU/µl. Transfer 1-5 µl to a well and bring the volume to 50 µl with Assy Buffer.

4. Start the reaction: Add 50 µl of the 5 mM pNPP solution to each well containing test samples or Positive Control.

5. Measure: Monitor the reaction progress using the absorbance at 405 nm of the samples and standards at 25°C for 15 - 60 minutes.

6. Typical Results:

	Standard Raw Values		Background Corrected Values	
nmol Standard	OD			
0	0.0522	0.0531	0.0063	0.0072
5	0.4002	0.4020	0.3543	0.3561
10	0.7782	0.7807	0.7323	0.7348
15	1.1262	1.1133	1.0803	1.0674
20	1.4942	1.4984	1.4483	1.4525
25	1.8322	1.8192	1.7863	1.7733



7. Calculations:
Standard: Correct background by subtracting the value derived from the 0 Standard from all Standards and samples. Plot the Standard curve. Determine the slope of the Standard Curve. The slope defines overall response in OD/nmol.

Test Samples: Select a time period for which the enzyme activity is linear. Determine the slope (OD/min) of the linear portion. Divide the slope of the enzyme activity (OD/min) by the slope of the Standard Curve (OD/nmol) to get the enzyme rate (nmol/minute) in the well. To convert that back to alkaline phosphatase activity in the original sample do the following:
A. Divide the AP activity in the well by the volume of sample added to the well = AP Activity (nmole/min/µl) of sample.
B. Multiply the AP Activity (nmole/min/µl) of sample X the total volume of supernatant recovered in step 2 above = total AP Activity per sample.
C. Divide the total AP Activity per sample by the mg of tissue or # of cells used to prepare the sample = AP Activity per mg tissue (or per # of cells, etc.

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