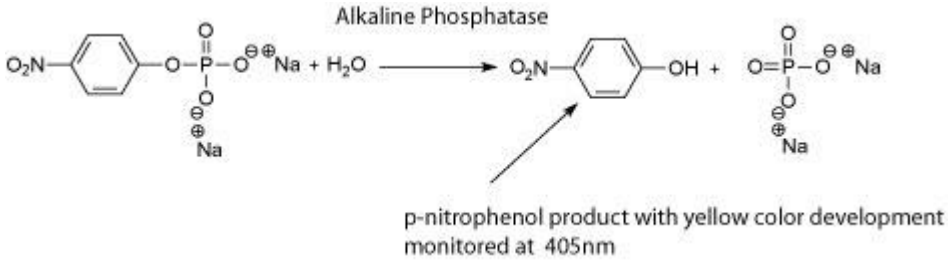
Rev. 1

Alkaline Phosphatase Activity Assay (ALP)
Catalog #8258
500 tests

Product Description
Protein phosphatase, an enzyme that controls the removal of phosphate (PO_4^{3-}) group from protein molecules, regulates many fundamental cellular processes such as cell attachment, proliferation, differentiation and apoptosis. Pluripotent stem cells typically have higher levels of alkaline phosphatase activity than differentiated cell types (although osteoblasts retain this activity). Alkaline phosphatase staining/activity is often used as a biomarker of stem cells. Alkaline Phosphatase Assay (ALP) is optimized to detect phosphatase activity in biological samples using pNPP (4-nitrophenyl phosphate) as a colorimetric substrate. A water-soluble yellow product with a strong absorption at 405 nm is developed during the reaction of pNPP with phosphatase and can be detected with a microplate reader.



Sensitivity Range
100 µUnit/mL - 100 mUnit/mL

Kit Components

Cat. No.	# of vials	Name	Quantity	Storage
8258a	1	Assay Buffer	25 ml	4°C
8258b	1	Enzyme (100 U/mL)	20 µl	-20°C
8258c	1	Substrate	2.5 ml	-20°C, in the dark
8258d	1	Cell Lysis Buffer	25 ml	4°C
8258e	1	Stop Solution	25 ml	4°C

Additional Materials Required (Not provided):
Microplate reader
96-well plates with clear flat-bottom
Dulbecco's Phosphate-Buffered Saline (Cat. #0303)

Quality Control
The activity of serially diluted enzyme phosphatases in provided assay buffer was measured with ALP after 15-60 minutes as shown in Figure 1.

Product Use
ALP is used to evaluate phosphatase activity *in vitro* and is for research use only. It is not approved for human or animal use or for applications in clinical or *in vitro* diagnostic procedures.

Shipping and Storage
Kit components are shipped on dry ice. Upon receipt store Cat.#8258a, 8258d and 8258e at 4ºC and 8258b and 8258c at -20ºC.

Procedure:
Reagent Preparation

1. **Assay Buffer**: Equilibrate to room temperature before use.
2. **Standard Preparation:** Always prepare a fresh set of standards for every use.
3. **Substrate**: Use 5 µl of substrate per 96 well reaction. Store aliquots in dark at -20°C.
4. **Stop Solution**: Equilibrate to room temperature before use.
5. **Lysis Buffer**: Ready to use. Equilibrate to room temperature before use.

Sample Preparation: Cell Lysate for intracellular ALP

1. Gently aspirate the cell culture medium (cell number may vary depending on the cell-type)
2. Wash the cells twice with Dulbecco's Phosphate-Buffered Saline (Catalog #0303).
3. Lyse the cells with appropriate amount of cell lysis buffer (0.5 ml for 35 mm dish).
4. Centrifuge the cell lysate at 14,000g for 5 minutes at 4ºC. Transfer the supernatant to a new tube.
5. Perform protein assay to determine total protein concentration in the lysate.
6. We recommend performing several dilutions in assay buffer to ensure your readings fall within the standard range.

Standard Preparation

1. Add 0.5 µl of Enzyme to 199.5 µl of assay buffer to make a 200 µl solution of 250 mU/ml enzyme standard.
2. Obtain 7 test tubes, add 150 µl of assay buffer into each tube and label them #1 through #7.
3. Add 150 µl of the 250 mU/ml enzyme standard into tube #1 and mix well to get the 125 mU/ml standard.
4. Transfer 150 µl of the 125 mU/ml enzyme standard from tube #1 to tube #2 and mix well to get the 62.5 mU/ml of standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the enzyme standards. Do not add any enzyme to tube #7, which serves as the blank.

#1	#2	#3	#4	#5	#6	#7
125 mU/ml	62.5 mU/ml	31.8 mU/ml	15.6 mU/ml	7.8 mU/ml	3.9 mU/ml	Blank

Assay Procedure

1. Apply 45 µl of each Standard/Sample/Blank in triplicate to each of a 96-well plate.
2. Add 5 µl of substrate per well and incubate in dark for 15-60 minutes.
3. Stop the reaction by adding 50 µl of stop buffer to each well. Mix and measure the absorbance on ELISA plate reader with a test wavelength at 405 and reference wave length at 630 nm. Determine the final absorbance value by performing the subtraction of the absorbance measured at 630 nm from that at 405 nm.

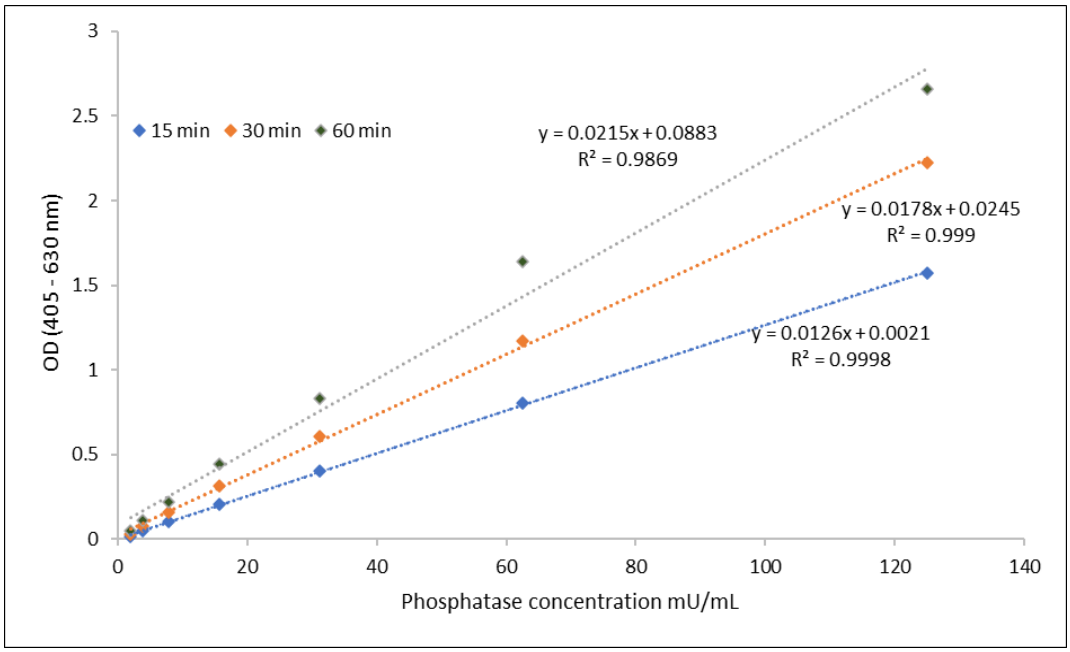


Figure 1. Alkaline phosphatase was serially diluted in Assay Buffer, and its activity was measured with ALP after a given time of reaction (15, 30 and 60 minutes).