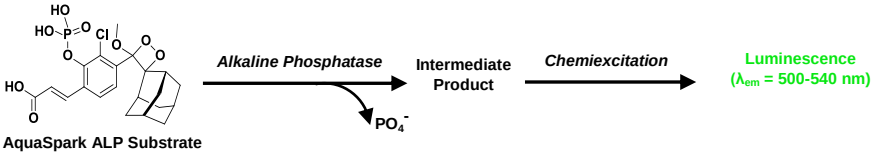
Alkaline Phosphatase Activity Assay Kit (Luminometric)

10/21

(Catalog # K2117-100; 100 Assays; Store at -20 °C)

I. Introduction:

Alkaline phosphatase(s) (ALP, EC 3.1.3.1) are a family of enzymes that are expressed in virtually all living organisms. The ALP superfamily has extremely broad substrate specificity, hydrolyzing many different chemically distinct phosphate esters to liberate inorganic phosphate. In humans, there are four ALP isozyme types: intestinal, placental, placental-like and tissue non-specific enzymes. The tissue non-specific form, in particular is highly expressed in pluripotent and multipotent stem cells such as bone-marrow derived mesenchymal stems cells. ALP is also a popular enzyme conjugate used to generate signal output in enzymatic immunoassays. **BioVision's Alkaline Phosphatase Activity Assay Kit** utilizes AquaSpark™, a water-soluble chemiluminescent dioxetane ALP substrate that enables the ultra-sensitive detection of ALP activity in various sample types. In this assay, the enzymatic cleavage of the phosphate ester moiety in the AquaSpark™ substrate yields an intermediate product that undergoes rapid self-immolative chemiexcitation, emitting bright green light (λ_{em} = 510 nm). The luminescent substrate has a high quantum yield in aqueous solutions and does not require any surfactants, enhancers or organic solvents to achieve maximum light emission. Because the AquaSpark™ luminescent probe is non-cytotoxic, the kit can also be used to identify pluripotent/multipotent stem cells or monitor osteogenic differentiation of mesenchymal stem cells without requiring lysis or fixation. The kit has a limit of detection of 0.005 µU/well of ALP activity in samples and can also be used for chemiluminescent detection in ELISAs with ALP-conjugated secondary antibodies.



II. Applications:

- Estimation of alkaline phosphatase activity in various biological samples
- Detection of alkaline phosphate activity in induced pluripotent and mesenchymal stem cells
- Conversion of ELISAs with ALP-conjugated secondary detection to chemiluminescence

III. Sample Types:

- Biological fluids
- Whole cells (e.g. multipotent/stem cells or mixed complex cultures)
- Lysates of cultured cells (adherent or suspension cells) and animal tissues
- ELISAs with an ALP-conjugated detection mechanism

IV. Kit Contents:

Components	K2117-100	Cap Code	Part Number
ALP Assay Buffer	25 ml	WM	K2117-100-1
AquaSpark™ ALP Substrate	100 µl	Red	K2117-100-2
ALP Lysis Buffer	25 ml	NM	K2117-100-3
ALP Positive Control	1 vial	White	K2117-100-4

V. User Supplied Reagents and Equipment:

- Multiwell fluorescence microplate reader
- 96-well white plate with flat bottom

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Allow the ALP Assay Buffer to warm to room temperature (RT) prior to use. Read the entire protocol before performing the assay procedure.

- **ALP Assay Buffer** and **ALP Lysis Buffer:** Allow to warm to RT prior to use. Store at +4 °C.
- **AquaSpark™ ALP Substrate:** Divide into aliquots and store at -20 °C, **protected from light and moisture**. Avoid repeated freeze-thaw cycles. Prior to use, warm solution to RT. **After use, promptly retighten the cap to minimize adsorption of airborne moisture.**
- **ALP Positive Control:** Reconstitute the vial in 1 ml of ALP Assay Buffer. Store at -20 °C and avoid repeated freeze-thaw cycles.

VII. Alkaline Phosphatase Activity Assay Protocol:

1. Sample Preparation:

For Whole Cells (Pluripotent/Multipotent Stem Cells) and Cell Lysates:

This assay was developed using human bone marrow-derived mesenchymal stem cells (BMSCs) as an example of multipotent cell line and Jurkat cells as an example of terminally-differentiated cell line. Cells were grown in T-25 sized culture dishes and seeded in a 96-well plate format at a density ranging from 1000 to 50,000 cells per well for ALP detection.

a. Culture cells to 80-90% confluence in appropriate growth, induction or differentiation medium according to the cell maintenance/differentiation protocol.

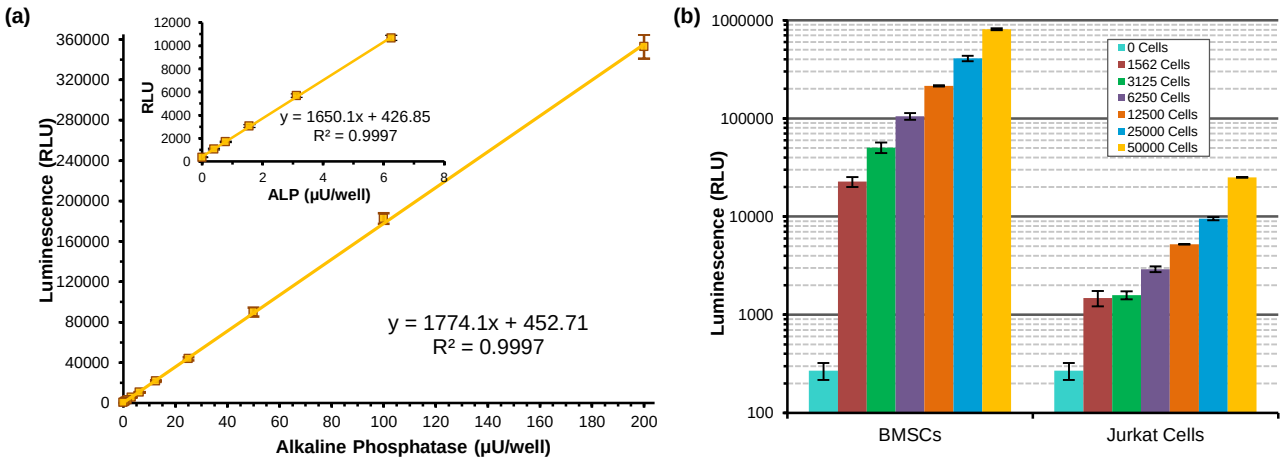
b. For adherent cells, detach using a cell dissociation solution (e.g. 0.25% trypsin or non-enzymatic dissociation solution) or manually with a rubber cell scraper and transfer cells to a clean microfuge tube. Rinse cells with **serum-free** medium and pellet by centrifugation at 600 x *g* and RT for 5 min. **For suspension cells,** pellet cells by centrifugation, rinse with serum-free medium and pellet again.

c. For detection of ***extracellular surface ALP only (whole cells)***, aspirate serum-free medium and resuspend cells in ALP Assay Buffer at a concentration of 1 x 10^6 cells per ml of buffer. For detection of ***total ALP activity (including intracellular)***, aspirate serum-free medium, resuspend in ALP Lysis Buffer (1 x 10^6 cells per ml of buffer), vortex thoroughly and incubate on ice for 15 min, vortexing every 5 min. Centrifuge lysate at 5000 x *g* for 10 min at 4 °C and transfer the supernatant to a clean microfuge tube. Add the sample volume that corresponds to the desired number of cells to sample wells (anywhere from 100-50000 whole cells or a corresponding volume of lysate) in an 96-well white plate and bring the volume up to 50 µl per well with ALP Assay Buffer.

For Tissues: Soft tissues (~10 mg wet tissue) should be homogenized on ice with 100 µl cold ALP Lysis Buffer using a Dounce tissue homogenizer (BioVision Cat# 1998) or probe sonicator. **Note:** Adjust the amount of ALP Lysis Buffer based upon the tissue sample weight). Centrifuge homogenate at 10,000 x *g* for 15 min at 4 °C and transfer the supernatant to a new micro-centrifuge tube. Add 2-50 µl of sample to desired wells in a 96-well white plate and bring the volume up to 50 µl per well with ALP Assay Buffer.

Background Control and **Positive Control:** In addition to sample wells, prepare a **Background Control** well by adding 50 µl of ALP Assay Buffer to a designated well. For **Positive Control**, dilute the reconstituted ALP Positive Control at 1:100 ratio with ALP Assay Buffer (i.e. mix 10 µl ALP Positive Control stock with 990 µl ALP Assay Buffer). Add 10 µl of the diluted ALP Positive Control to a designated well and bring the volume up to 50 µl with ALP Assay Buffer.

2. **Reaction Mix Preparation:** For each reaction well (including **Background Control** and **Positive Control** wells, if applicable), prepare 50 µl of Reaction Mix by mixing 1 µl of AquaSpark™ ALP Probe and 49 µl ALP Assay Buffer. Prepare sufficient amount of the Reaction Mix for all of the assay wells. **Note:** We recommend preparing a minimum of 5 reaction wells to ensure pipetting accuracy. Add 50 µl Reaction Mix to each reaction well including Samples(s), Background Control and Positive Control. Mix the reagents completely by gently tapping the plate.
3. **Measurement:** Start measuring the luminescence intensity in kinetic mode, reading luminescence every 1-2 min for at least 30 min. Note that the peak luminescence is typically achieved within 5-10 min and the signal intensity begins to decay after 15-30 min, depending upon the sample ALP activity.
4. **Calculation:** Calculate the net luminescence signal (*L*) by subtracting the Background Control (BC) RLU reading from each of the corresponding Sample RLU readings: $L = RLU_{Sample} - RFU_{BC}$.



Figures: (a) Luminescence detection of alkaline phosphatase activity using AquaSpark™ ALP Substrate (detection of less than 1 µU enzyme as shown in inset graph). One unit of alkaline phosphatase activity is defined as hydrolysis of 1 µmole of the colorimetric substrate 4-nitrophenylphosphate per minute at pH 9.6 in a glycine-based buffer. LOD for the assay is 0.0048 µU/well, data are mean ± SD of triplicate wells. **(b)** Detection of alkaline phosphatase activity in whole cells, comparing a multipotent cell line (human bone-marrow derived mesenchymal stem cells; BMSCs) versus a terminally differentiated cell line (Jurkat cells). The data represent mean RLU values at 10 minutes after addition of the AquaSpark™ ALP Probe (absolute peak luminescence occurred between 7 and 9 minutes after probe addition). Data are mean ± SD of triplicate wells, each containing the indicated number of cells.

VIII. Related Products:

Alkaline Phosphatase Activity Colorimetric Assay Kit (K412)
Alkaline Phosphatase Staining Kit (K2035)
Phytase Activity Assay Kit (K2054)
Alkaline Phosphatase Activity Fluorometric Assay Kit (K422)
Acid Phosphatase Activity Colorimetric Assay Kit (K412)
Protein Tyrosine Phosphatase Activity Assay Kit (K829)

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