ab307272 – Alkaline Phosphatase Activity Assay Kit (Luminometric)

Detection of alkaline phosphate activity in induced pluripotent and mesenchymal stem cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

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.

Materials Supplied

Item	Quantity	Storage Condition
ALP Assay Buffer	25 ml	4 °C
ALP Substrate (Avoid light)	100 µl	-20 °C
ALP Lysis Buffer	25 ml	4 °C
ALP Positive Control	1 vial	-20 °C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

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.

Alkaline Phosphatase Activity Assay Protocol

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.

1. Culture cells to 80-90% confluence in appropriate growth, induction or differentiation medium according to the cell maintenance/differentiation protocol.
2. 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.
3. 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, vortex 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 a 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 or probe Sonicator.

Δ 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.*

1.

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.

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 Substrate and 49 µl ALP Assay Buffer. Prepare sufficient amount of the Reaction Mix for all of the assay wells.

Δ 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.

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.

Calculation:

Calculate the net luminescence signal (*L*) by subtracting the Background Control (BC) RLU reading from each of the corresponding Sample RLU readings: $L = RLU_{Sample} - RFU_{BC}$.

Technical Support

For all technical or commercial enquiries please go to:
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