

Datasheet for ABIN1113908

Intestinal Alkaline Phosphatase ELISA Kit



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Overview

Quantity:	96 tests
Target:	Intestinal Alkaline Phosphatase (ALPI)
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	0.78 ng/mL - 50 ng/mL
Minimum Detection Limit:	0.78 ng/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay technique for the in vitro quantitative measurement in various sample types.
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This kit recognizes natural and recombinant Rat ALPI. No significant cross-reactivity or interference between Rat ALPI and analogues was observed. Note: Limited by existing techniques, cross reaction may still exist, as it is impossible for us to complete the cross-reactivity detection between Rat ALPI and all the analogues.
Cross-Reactivity (Details):	No significant cross-reactivity or interference between rat Calf Intestine Alkaline Phosphatase and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between Human bFGF and all the analogues,

Product Details

therefore, cross reaction may still exist.

Sensitivity: 0.47 ng/mL

Components:

- Pre-coated, ready to use 96-well strip plate, flat bottom
- Plate sealer for 96 wells
- Reference Standard
- Reference Standard & Sample Diluent
- Biotinylated Detection Antibody (100 x concentrate)
- HRP Conjugate (100 x concentrate)
- Biotinylated Detection Antibody Diluent
- HRP Conjugate Diluent
- Substrate Reagent
- Stop Solution
- Wash Buffer (25 x concentrate)
- Instruction manual

Target Details

Target: Intestinal Alkaline Phosphatase (ALPI)

Alternative Name: ALPI(Alkaline Phosphatase, Intestinal) ([ALPI Products](#))

Application Details

Application Notes:

ELISA Plate: The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experiment results.

Add Sample: The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. Parallel measurement is recommended.

Incubation: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature.

Washing: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be pat dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note that clear the residual liquid and fingerprint in the bottom before measurement, so as not to affect the micro-titer plate reader.

Reagent Preparation: As the volume of Detection Ab and HRP Conjugate is very small, liquid may adhere to the tube wall or tube cap when being transported. You better hand-throw it or centrifugal it for 1 minute at 1000rpm. Please pipette the solution for 4-5 times before pipetting. Please carefully reconstitute Standards, working solutions of Detection Ab and HRP Conjugate according to the instructions. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10µL for once pipetting. Do not reuse standard solution, working solution of Detection Ab and HRP Conjugate, which have been diluted. If you need to use standard repeatedly, you can divide the standard into small pack according to the amount of each assay, keep them at -20°C to -80°C and avoid repeated freezing and thawing.

Reaction Time Control: Please control reaction time strictly following this product description!

Substrate: Substrate Solution is easily contaminated. Please protect it from light. **Stop Solution:** As it is an acid solution, please pay attention to the protection of your eyes, hands, face and clothes when using this solution.

Mixing: You'd better use micro-oscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no micro-oscillator available, you can knock the ELISA plate frame gently with your finger before reaction.

Security: Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.

Do not use component from different batches of kit(washing buffer and stop solution can be an exception)

To avoid cross-contamination, change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent. Otherwise, the results will be inaccurate!

Comment:

Information on standard material:

The formulation of the standard is 0.01 M PBS. The standard contains additives (1 % BSA).

Information on reagents:

Reagents include 1 M SO₂. Azide, thimerosal, 2-mercaptoethanol (2-ME) or any other poisonous materials are not used.

Information on antibodies:

The provided antibodies and their host vary in different kits. All antibodies are affinity purified

Application Details

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Sample Volume: 100 µL

Plate: Pre-coated

Protocol:

1. Add 100 µL standard or sample to each well. Incubate for 90 min at 37 °C.
2. Remove the liquid. Add 100 µL Biotinylated Detection Antibody. Incubate for 1 hour at 37 °C.
3. Aspirate and wash 3 times.
4. Add 100 µL HRP Conjugate. Incubate for 30 min at 37 °C.
5. Aspirate and wash 5 times.
6. Add 90 µL Substrate Reagent. Incubate for 15 min at 37 °C.
7. Add 50 µL Stop Solution. Read at 450 nm immediately.
8. Calculation of results.

Reagent Preparation:

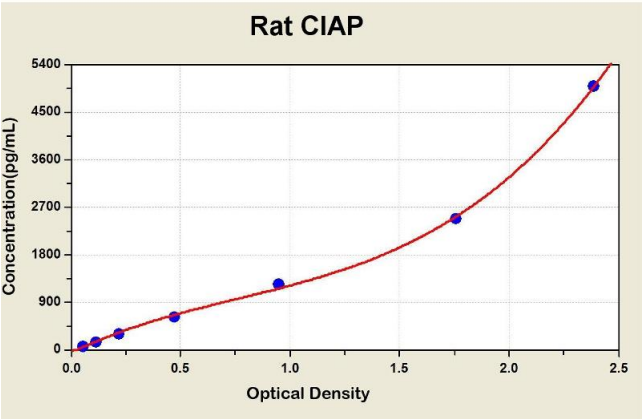
1. Bring all reagents to room temperature (18~25 °C) before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.
2. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40 °C water bath and mix it gently until the crystals have completely dissolved.
3. Standard working solution: Centrifuge the standard at 10,000xg for 1 min. Add 1.0 mL of Reference Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 50 ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 50, 25, 12.5, 6.25, 3.13, 1.57, 0.78, 0 ng/mL. Dilution method: Take 7 EP tubes, add 500 µL of Reference Standard & Sample Diluent to each tube. Pipette 500 µL of the 50 ng/mL working solution to the first tube and mix up to produce a 25 ng/mL working solution. Pipette 500 µL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.
4. Biotinylated Detection Antibody working solution: Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100x Concentrated Biotinylated Detection Antibody to 1x working solution with Biotinylated Detection Antibody Diluent.
5. Concentrated HRP Conjugate working solution: Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100x Concentrated HRP Conjugate to 1x working solution with Concentrated HRP Conjugate Diluent.

Restrictions: For Research Use only

Handling

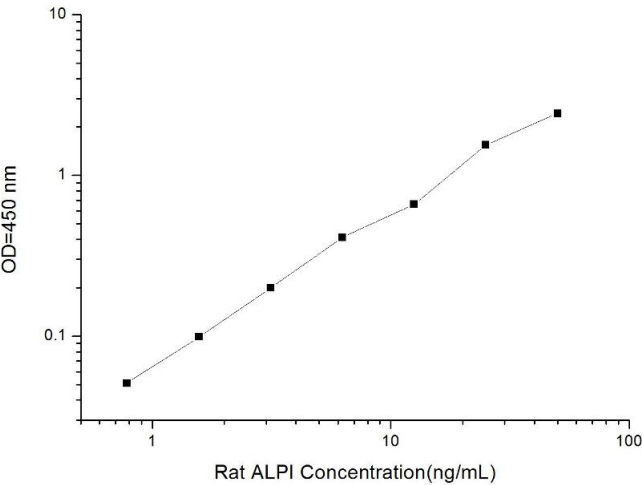
Handling Advice:	All the reagents in the kit should be stored according to the labels on vials. Unused wells should be returned to the foil pouch with the desiccant pack and resealed along entire edge of zip-seal. Substrate Reagent shouldn't be kept at -20 °C (Check!). Exposure of reagents to strong light should be avoided in the process of incubation and storage. All the taps of reagents should be tightened to prevent evaporation and microbial contamination. If not to store reagents according to above suggestions, erroneous results may occur.
Storage:	4 °C/-20 °C
Storage Comment:	The unopened kit can be stored at 4°C for 1 month. <i>If the kit is not used within 1 month, store the items separately according to the conditions since the kit is received.</i>

Images



ELISA

Image 1. Diagramm of the ELISA kit to detect Rat C1 APwith the optical density on the x-axis and the concentration on the y-axis.



ELISA

Image 2. Typical standard curve