Pyrophosphate Assay kit

ARG83378 Pyrophosphate Assay kit is an assay kit for Pyrophosphate in Serum, plasma, saliva, urine, Cell culture supernatants, cell lysate and tissue lysates.

Catalog number: ARG83378

Package: 100 assay

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INTRODUCTION

In chemistry, pyrophosphates are phosphorus oxyanions that contain two phosphorus atoms in a P–O–P linkage. A number of pyrophosphate salts exist, such as disodium pyrophosphate (Na2H2P2O7) and tetrasodium pyrophosphate (Na4P2O7), among others. Often pyrophosphates are called diphosphates. The parent pyrophosphates are derived from partial or complete neutralization of pyrophosphoric acid. The pyrophosphate bond is also sometimes referred to as a phosphoanhydride bond, a naming convention which emphasizes the loss of water that occurs when two phosphates form a new P–O–P bond, and which mirrors the nomenclature for anhydrides of carboxylic acids. Pyrophosphates are found in ATP and other nucleotide triphosphates, which are important in biochemistry. The term pyrophosphate is also the name of esters formed by the condensation of a phosphorylated biological compound with inorganic phosphate, as for dimethylallyl pyrophosphate. This bond is also referred to as a high-energy phosphate bond.

PRINCIPLE OF THE ASSAY

ARG83378 Pyrophosphate Assay Kit measures Pyrophosphate content in biological samples. Pyrophosphate and phosphoenolpyruvate is converted by PPDK to pyruvate + adenosine triphosphate (ATP) and phosphate. Pyruvate is converted by pyruvate oxidase in the presence of phosphate and oxygen into acetyl phosphate, carbon dioxide, and hydrogen peroxide. Samples are compared to a known concentration of Pyrophosphate standard. The intensity of the color is measured at a wavelength of 540 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store 10X Assay Buffer at RT, Store PPDK at -80°C Store other component at ≤ -20°C. Use the kit before expiration date.

Component	Quantity	Storage information
Standard (25 mM)	50 µL	-20°C
10X Assay Buffer	25 mL	RT
Phosphate pyruvate dikinase (PPDK)	200 µL	-80°C
Adenosine monophosphate (AMP)	50 µL	-20°C
Phosphoenolpyruvate (PEP)	50 µL	-20°C
Sodium phosphate dibasic (Na2HPO4)	50 µL	-20°C
Probe	50 µL	-20°C
HRP-Streptavidin Solution	10 µL	-20°C
Flavin Adenine Dinucleotide (FAD)	50 µL	-20°C
Thiamine Pyrophosphate (TPP)	50 µL	-20°C
Pyruvate Oxidase	300 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540 nm
- Flat bottomed 96-well black microplate and tube.
- 1X PBS and deionized water
- Pipettes and pipette tips
- 10 kDa molecular weight cutoff spin filter

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Upon received, store 10X Assay Buffer at RT, Store PPDK at -80°C Store other component at ≤ -20°C. Use the kit before expiration date and avoid freeze / thaws.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to 4°C / room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Saliva- Collect saliva using a collection device (e.g. Salivette), centrifuge 10,000 x g for 2 min at 4°C. Collect saliva and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles. The collection device should not have protein binding or filtering features.

Urine - Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at-80°C. Perform dilutions in PBS.

REAGENT PREPARATION

- **1X Assay Buffer:** Dilute the 10X Assay Buffer with deionized water to yield 1X Assay Buffer. Store at RT.

- **Reaction Mix and Control Mix:**

  Prepare two separate mixtures according to the table below.

Component	Reaction Mix (20 assays)	Negative Control Mix (20 assays)
PPDK	40 µL	-
AMP	10 µL	10 µL
PEP	10 µL	10 µL
HRP	2 µL	2 µL
Pyruvate Oxidase	60 µL	60 µL
FAD	10 µL	10 µL
TPP	10 µL	10 µL
Na2HPO4	10 µL	10 µL
Probe	10 µL	10 µL
1X Assay Buffer	838 µL	878 µL
Total	**1000 µL**	**1000 µL**

- **Standards**: Add 5 µl of 25.0 mM stock standard into 995 µl 1X Assay Buffer to generate a standard with 125 µM of Pyrophosphate. Dilute the standards with 1X Assay Buffer serves as zero standard (blank standard, 0 µM). The example of the standards dilution table is as below:

Standard	Pyrophosphate (µM)	Volume of 1X Assay Buffer (µL)	Volume of Pyrophosphate (µL)
S1	125	995	5 (25.0 mM stock)
S2	62.5	250	250(S1)
S3	31.25	250	250(S2)
S4	15.63	250	250(S3)
S5	7.81	250	250(S4)
S6	3.91	250	250(S5)
S8	0	250	0

ASSAY PROCEDURE

Each samples should be assayed in at least duplicates, one to be treated with PPDK and one without, to measure endogenous background.

1. Add **50 µl** of **standard** and **sample** to each wells.
2. Add **50 µL** of **Reaction Mix** to **standard** and half of sample wells**.**
3. Add **50 µL** of **Negative Control Mix** to other half of sample wells.
4. Mix well and Incubate for 30 min at 37°C.

   Note: This assay is continuous (not terminated), therefore may be measured at multiple time points to follow the reaction kinetics.
5. Read O.D. with a microplate reader at **590 nm** immediately.

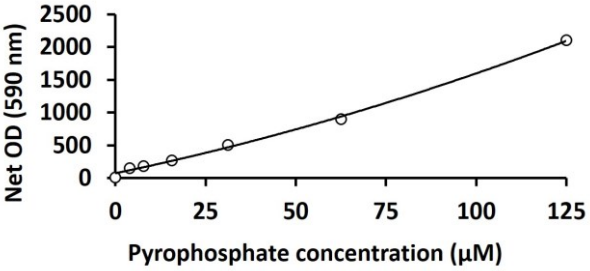
CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of standards and samples.
2. Subtract the average value of Standard 0 from all standard value.
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
4. Subtract the sample well values without PPDK from the sample well values containing PPDK (Control Mix) to obtain the difference. The absorbance difference is due to the L-Pyrophosphate Dehydrogenase activity:

   $\Delta A = A_{\text{Reaction Mix}} - A_{\text{Negative Control Mix}}$
5. Compare the change in absorbance Δ A of each sample to the standard curve to determine and extrapolate the quantity of bile acid present in the sample. Only use values within the range of the standard curve.

EXAMPLE OF TYPICAL STANDARD CURVE

The following table shows the OD readings of a run of this assay kit with serial diluted standards



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Pyrophosphate ranged from 3.9-125 µM. The mean MDD was 2.5 µM