Version 3, Last updated 23 November 2023

ab234040 Pyrophosphate Assay Kit (Colorimetric / Fluorometric)

For the measurement of Pyrophosphate in cell culture extracts, tissue lysates, plasma, serum and other biological fluids.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

Pyrophosphate Assay Kit (Colorimetric / Fluorometric) (ab234040) provides a fast, convenient and ultrasensitive method for determination of free inorganic pyrophosphate (PPi) levels in biological material.

PPi produced during biotic processes is detected through a series of reactions which utilize a proprietary enzyme mix and probe, generating a stable product that can be quantified by either colorimetric or fluorometric readout. Generated fluorescence (Ex/Em = 535/587 nm) or color (OD 570 nm) intensities are directly proportional to the concentrations of pyrophosphate, enabling precise measurements. Monomeric inorganic phosphate (Pi) does not interfere with the assay.

This kit delivers an easy and robust method suitable for use in a variety of biological samples and can be performed in a convenient microtiter-plate format. The kit provides sufficient reagents for 100 fluorometric or 50 colorimetric assays, respectively. This kit can detect as low as 1.8 µM PPi in plasma and serum samples

Prepare samples.

⇩

Prepare standard curve.

⇩

Prepare reaction mix.

⇩

Add samples, controls and standards to wells and incubate plate protected from light.

⇩

Measure absorbance (570 nm) or fluorescence EX/Em = 535/587 nm).

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer XV/PPi Assay Buffer	25 ml	-20°C	-20°C
AMP/PPi Buffer Supplement	200 µl	-20°C	-20°C
Development Enzyme Mix I/PPi Developer	1 vial	-20°C	-20°C
Enzyme Mix X/PPi Enzyme Mix	200 µl	-20°C	-20°C
OxiRed Probe/PPi Probe	200 µl	-20°C	-20°C
Pyrophosphate Standard /PPi Standard (1 mM)	200 µl	-20°C	-20°C
PEP/PPi Substrate	2 vials	-20°C	-20°C

3. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at OD 570 nm or fluorescence at Ex/Em = 535/587 nm
- 96 well plate with clear flat bottom (for colorimetric assay) / 96 well plate with clear flat bottom, preferably black (for fluorometric assay)
- Dounce homogenizer (if using tissue)
- 10 kDa spin columns for sample preparation

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Assay Buffer XV/PPi Assay Buffer

Ready to use as supplied. Warm to RT before use. Store at -20°C. Stable for one month.

5.2 AMP/PPi Buffer Supplement

Ready to use as supplied. Store at -20°C, thaw and keep on ice while using.

5.3 Development Enzyme Mix I/PPi Developer

Completely dissolve with 220 µl of Assay Buffer XV/PPi Assay Buffer, aliquot and store at -20°C. Use within one month.

5.4 Enzyme Mix X/PPi Enzyme Mix

Ready to use as supplied. Store at -20°C, thaw and keep on ice while using.

5.5 OxiRed Probe/PPi Probe

Ready to use as supplied. Warm to RT before use. Store at -20°C. Stable for one month.

5.6 Pyrophosphate Standard/PPi Standard (1 mM)

Ready to use as supplied. Warm to RT before use. Store at -20°C. Stable for one month.

5.7 PEP/PPi Substrate

Completely dissolve one vial with 100 µl of ddH_2O and store at -20°C. Use within one month.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

For colorimetric detection:

1. Using Pyrophosphate Standard /PPi Standard (1 mM), prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	PPi Standard (1 mM) (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount of PPi (nmol/well)
1	0	100	50	0
2	4	96	50	2
3	8	92	50	4
4	12	88	50	6
5	16	84	50	8
6	20	80	50	10

Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

For fluorometric detection:

1. For assays ranging between 0-1 nmol PPi: Dilute the 1 mM Pyrophosphate Standard/PPi Standard at a 1:10 ratio in Assay Buffer XV/PPi Assay Buffer to obtain a 100 µM Pyrophosphate Standard/PPi Standard working solution.
2. For assays ranging between 0-0.1 nmol PPi: Generate a 10 µM Pyrophosphate Standard/PPi Standard solution by further diluting the 100 µM working solution at 1:10 ratio.
3. Using the 100 µM or 10 µM Pyrophosphate Standard/PPi standard, prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tubes.
4. Add 0, 2, 4, 6, 8, and 10 µl of either the 100 µM or the 10 µM Pyrophosphate Standard/PPi Standard solution into a series of wells, generating a standard curve of either 0, 200, 400, 600, 800 and 1000 or 0, 20, 40, 60, 80 and 100 pmol/well of Pyrophosphate Standard/PPi Standard, respectively.

Standard #	PPi Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount of PPi in well (pmol/well)
1	0	100	50	0/0
2	4	96	50	200/20
3	8	92	50	400/40
4	12	88	50	600/60
5	16	84	50	800/80
6	20	80	50	1000/100

Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

7. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.
- If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware, however, that this might affect the stability of your samples and the readings can be lower than expected. Avoid multiple freeze-thaws.
- Since endogenous compounds may interfere with the reaction, to ensure accurate measurement of PPi in the test wells, we recommend spiking the samples with a known amount of Pyrophosphate Standard/PPi Standard within the standard curve range.

7.1 Blood, plasma and serum samples:

1. Centrifuge blood, plasma and serum samples for 10 minutes at 10,000 x *g* and 4°C and collect the supernatant.
2. Filter pre-cleared supernatant through a 10 kDa spin column (10,000 x *g* at 4°C for 10 minutes) and use the deproteinized filtrate for analysis.
3. Add 2-50 µL of sample into a clear 96-well plate and adjust the volume to 50 µL with Assay Buffer XV/PPi Assay Buffer.

7.2 Cells and tissue extracts/ lysates:

1. Cells and tissues can be extracted directly in Assay Buffer XV/PPi Assay Buffer by mechanical disruption, liquid homogenization, sonication, freeze/thaw cycles, manual grinding, or lysed by your method of choice.
2. Add 2-50 µL of sample into a clear 96-well plate and adjust the volume to 50 µL with Assay Buffer XV/PPi Assay Buffer.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

Δ Note: If you suspect your samples contain substances that can generate significant background, set up Sample Background Controls to correct for background noise.

Δ Note: For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

8.1 Reaction wells set up:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer XV/PPi Assay Buffer).
- Sample Background Control wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer XV/PPi Assay Buffer).

8.2 PPi Reaction mix:

1. Prepare 50 µL of Reaction Mix and Background Mix for each reaction. Prepare a master mix to ensure consistency.

	Fluorometric assay		Colorimetric assay	
Component	Reaction Mix (µL)	Background Reaction Mix (µL)	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer XV/PPi Assay Buffer	40	42	30	34
AMP/PPi Buffer Supplement	2	2	4	4
PEP/PPi Substrate	2	2	4	4
Enzyme Mix X/PPi Enzyme Mix	2	-	4	-

Development Enzyme Mix I/PPi Developer	2	2	4	4
OxiRed Probe/PPi Probe	2	2	4	4

2. Add 50 µL of Reaction Mix into each standard and sample wells.
3. Add 50 µL of Background Reaction Mix into the background control sample wells.
4. **Measurement:** For colorimetric assays, incubate the plate, protected from light, for 30 minutes at 37°C and measure absorbance (OD) at 570 nm.
5. **Measurement**: For fluorometric assays, incubate the plate for 60 minutes at 37°C, protected from light, and measure fluorescence at Ex/Em = 535/587 nm in endpoint mode.

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (zero PPi) from all standards controls and sample readings. This is the corrected absorbance/fluorescence.
3. If significant, subtract the sample background control from sample readings.
4. Plot the corrected values for each standard as a function of the final amount of Pyrophosphate.
5. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
6. Apply the corrected sample OD/RFU reading to the standard curve to get Pyrophosphate (B) amount in the sample wells.
7. Concentration of Pyrophosphate in B nmol / V µL in the test samples is calculated as:

$$\text{Pyrophosphate concentration} = \frac{B}{V} * D = \text{nmol}/\mu\text{L} = \text{mM}$$

Where:

B = amount of Pyrophosphate in the sample well calculated from standard curve in nmol.

V = sample volume added in the sample wells in µL.

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

In case of spiked samples use the following equation, wherever required;

8. If using **spiked samples**, correct for any sample matrix interference by subtracting the sample reading from the spiked sample reading. This equation allows you to measure the Pyrophosphate concentration in your sample when matrix interference is significant.

$$B = \left(\frac{(Sample\ corrected)}{(Spiked\ corrected) - (Sample\ corrected)}\right) * PPi\ Spike\ (nmol)$$

Where:

B = PPi amount in sample well (nmol)

Sample corrected = OD/RFU of sample with blank and background readings subtracted

Spiked corrected = OD/RFU of spiked sample with blank and background readings subtracted

PPi Spike = amount of PPi spiked (nmol) into the sample well

PPi MW = 446.06 g/mol (1 nmol PPi = 446.06 ng)

10. FAQs/Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

11. Typical Data

Data provided **for demonstration purposes only**.

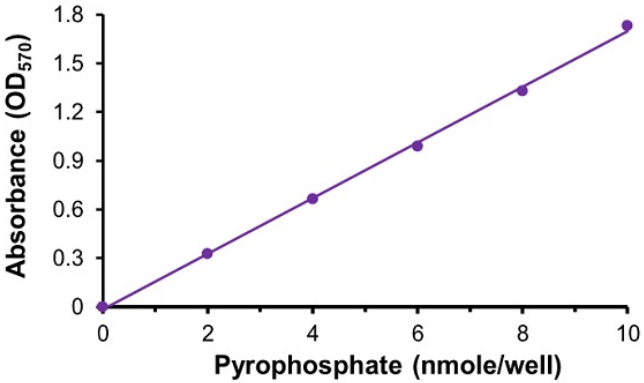

Figure 1. Example Colorimetric Standard Curve

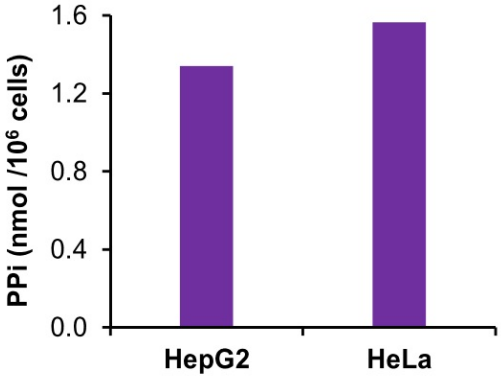

Figure 2. Pyrophosphate measured in HepG2 and HeLa cell lysates. Cells were extracted directly in the Assay Buffer.

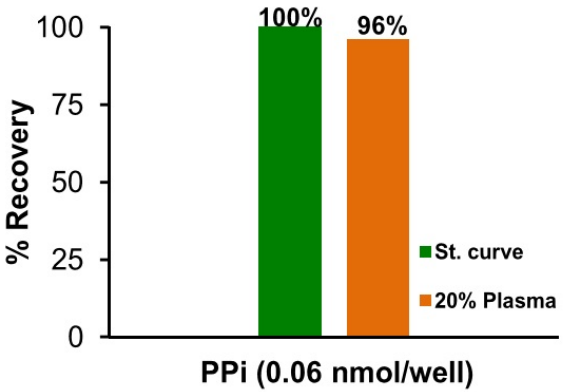

Figure 3. Spike and recovery in 20 µl of normal human plasma. Plasma samples were spiked with 0.06 nmol of Pyrophosphate Standard/PPi Standard and assayed according to kit protocol yielding 96% PPi recovery.

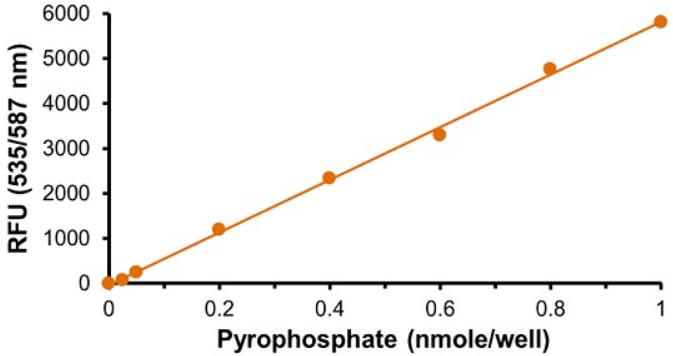

Figure 4. Example Fluorometric Standard Curve.

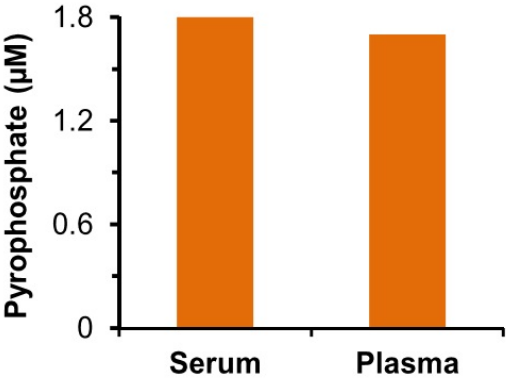

Figure 5. Quantification of pyrophosphate in 20 µL of de-proteinized undiluted human serum and pooled plasma.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)