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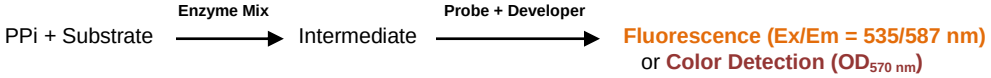
Pyrophosphate (PPi) Assay Kit (Fluorometric/Colorimetric)

10/17

(Catalog #K568-100; 100 Fluorometric or 50 Colorimetric assays; Store at -20°C)

I. Introduction:

Inorganic pyrophosphate (diphosphate, pyrophosphoric acid or PPi) is a small molecule formed during, or required as a substrate for, a number of biochemical reactions. Enzymatic reactions that generate PPi include ATP hydrolysis, DNA and RNA polymerization, cyclic AMP formation, and enzymatic activation of fatty acids to form their CoA esters. Conversely, enzymes that utilize PPi may include cyclases, hydrolases and ligases. Measurement of inorganic pyrophosphate (PPi) in plasma, serum and other biologic fluids is important in studies of bone metabolism, renal stone disease, and certain types of arthritis. BioVision's Pyrophosphate (PPi) Assay Kit provides a fast, convenient and ultrasensitive method for determination of free inorganic pyrophosphate 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. Our assay 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.



II. Applications:

- Determination of PPi concentration in biological samples
- Monitor pyrophosphate release by a variety of enzymes
- Screening inhibition or activities of enzymes that consume pyrophosphate

III. Sample Type:

- Plasma, serum and other biological fluids
- Cell culture extracts and tissue lysates

IV. Kit Contents:

Components	K568-100	Cap Code	Part Number
PPi Assay Buffer	25 ml	WM	K568-100-1
PPi Buffer Supplement	200 µl	Clear	K568-100-2
PPi Substrate	2 vials	Yellow	K568-100-3
PPi Enzyme Mix	200 µl	Blue	K568-100-4
PPi Developer	1 vial	Green	K568-100-5
PPi Probe	200 µl	Red	K568-100-6
PPi Standard (1 mM)	200 µl	Orange	K568-100-7

V. User Supplied Reagents and Equipment:

- Plate Reader capable of 37°C temperature setting and readings of fluorescence (Ex/Em 535/587 nm) or absorbance (570 nm)
- 96-well clear plates with flat bottom
- 10 kDa spin columns (Cat. #1997 or equivalent) for sample preparation

VI. Storage Conditions and Reagent Preparation:

Upon receiving store the entire kit at -20°C protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **PPi Buffer Supplement and PPi Enzyme Mix**: Store at -20°C, thaw and keep on ice while using.
- **PPi Substrate**: Completely dissolve one vial with 100 µl of ddH_2O and store at -20°C. Use within one month.
- **PPi Developer:** Completely dissolve with 220 µl of PPi Assay Buffer, aliquot and store at -20°C. Use within one month.
- **PPi Assay Buffer, PPi Probe and PPi Standard (1 mM):** Warm to RT before use. Store at -20°C. Stable for one month.

VII. Pyrophosphate (PPi) Assay Kit Protocol:

1. **Sample Preparation:** Centrifuge blood, plasma and serum samples for 10 min at 10000 x *g* and 4°C and collect the supernatant. Filter pre-cleared supernatant through a 10 kDa MWCO spin column (10000 x *g* at 4°C for 10 min; Cat. #1997) and use the deproteinized filtrate for analysis. Cells and tissues can be extracted directly in PPi Assay Buffer by mechanical disruption, liquid homogenization, sonication, freeze/thaw cycles, manual grinding, or lysed by your method of choice. Add 2-50 µl of sample into a clear 96-well plate and adjust the volume to 50 µl with PPi Assay Buffer.
   Notes:
   - For samples with significant background, prepare parallel reactions containing the same amount of sample as in the test wells. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
   - Since endogenous compounds might interfere with the reaction, to ensure accurate measurement of PPi in the test wells, we recommend spiking the samples with known amount of PPi Standard within the standard curve range.
2. **Standard Curve Preparation:** For **colorimetric** detection, use undiluted PPi Standard (1 mM). Add 0, 2, 4, 6, 8, and 10 µl of PPi Standard into a series of wells in and adjust the volume to 50 µl per well with PPi Assay Buffer. This will generate 0, 2, 4, 6, 8, 10

nmol/well of PPi Standard. For **fluorometric** detection in the *0-1 nmol range*, dilute the 1 mM PPi Standard at a 1:10 ratio in PPi Assay Buffer to obtain a 100 µM PPi Standard working solution. For assays ranging between *0-0.1 nmol PPi*, generate a 10 µM PPi Standard solution by further diluting the 100 µM working solution at 1:10 ratio. Add 0, 2, 4, 6, 8, and 10 µl of either the 100 µM or the 10 µM 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 PPi Standard, respectively.

3. **Reaction Mix:** Mix enough reagents for the number of reactions to be performed according to the table below (remember to account for standard curve wells). For standard curve and sample wells prepare 50 µl Reaction Mix, and for sample background wells 50 µl Background Control Mix respectively. Mix well by pipetting up and down before use.

	Fluorometric Assay		***Colorimetric Assay***	
	Reaction Mix	****Background Control Mix***	***Reaction Mix***	****Background Control Mix***
PPi Assay Buffer	40 µl	42 µl	30 µl	34 µl
PPi Buffer Supplement	2 µl	2 µl	4 µl	4 µl
PPi Substrate	2 µl	2 µl	4 µl	4 µl
PPi Enzyme Mix	2 µl	—	4 µl	—
PPi Developer	2 µl	2 µl	4 µl	4 µl
PPi Probe	2 µl	2 µl	4 µl	4 µl

*Add 50 µl Reaction Mix and 50 µl Background Control Mix to their respective 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. 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.

5. **Calculation:** Subtract the 0 PPi standard reading from all the standard readings and plot the PPi standard curve. If sample background control values are significant then subtract the sample background control reading from sample readings. For unspiked samples, apply the background-corrected absorbance/fluorescence values to the standard curve to obtain **B** nmol of PPi in the sample well:

Pyrophosphate (PPi) Concentration = B/V × D = nmol/µl ≡ mM

Where: **B** is PPi amount from the standard curve (in nmol)
V is Volume of the sample used in the reaction (in µl)
D is Sample Dilution Factor (if applicable)

Note: For spiked samples, calculate **B** by subtracting the background-corrected sample reading from corrected spiked sample reading

$$\textbf{PPi amount in spiked-sample wells (B)} = \left(\frac{\textbf{Sample(corrected)}}{(\textbf{Sample+PPi Spike(corrected)})-(\textbf{Sample(corrected)})}\right) \times \textbf{PPi Spike (in nmol)}$$

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

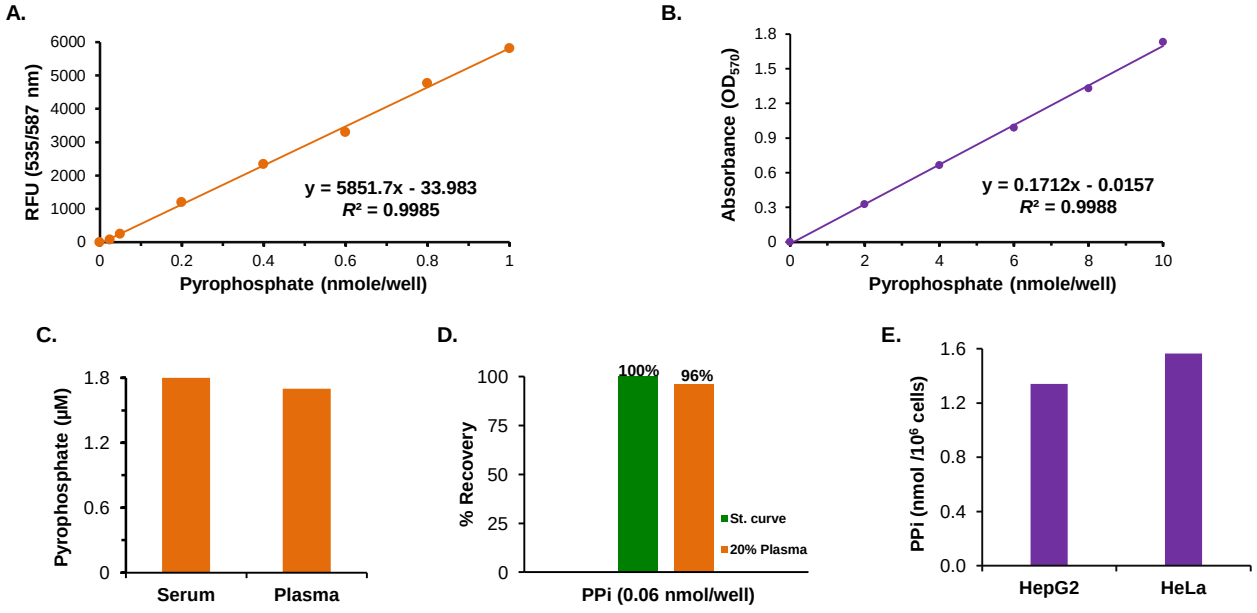


Figure: Pyrophosphate (PPi) Standard Curves: Fluorometric **(A)** and Colorimetric **(B)**. Quantification of pyrophosphate in 20 µl de-proteinized undiluted human serum and pooled plasma **(C)**. Spike and recovery in 20 µl of normal human plasma. Plasma samples were spiked with 0.06 nmol of PPi Standard and assayed according to kit protocol yielding 96% PPi recovery **(D)**. Pyrophosphate measured in HepG2 and HeLa cell lysates according to kit protocol. Cells were extracted directly in the Assay Buffer **(E).**

VIII. RELATED PRODUCTS:

Phosphate Assay Kit (Fluorometric) (K420)
Phosphate Colorimetric Assay Kit (K410)
ATP Colorimetric/Fluorometric Assay Kit (K354)
DNAse I Activity Assay Kit (Fluorometric) (K429)

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