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Inorganic Polyphosphate Assay Kit (Fluorometric)

12/19

(Catalog # K2025-100; 100 assays, Store kit at -20°C)

I. Introduction:

Inorganic polyphosphate (Poly P) is a linear polymer of hundreds of orthophosphate (Pi) residues linked by high-energy, phosphoanhydride bonds. Poly P is ubiquitous and can be found in all living organisms from bacteria to mammals. Poly P has many important cellular functions. In microorganisms, Poly P plays an important role in virulence, biofilm formation, motility, quorum sensing, stress response and survival during nutrient deficiency. In mammals, it is important for blood coagulation, calcium precipitation, immune response, apoptosis, signal transduction and mitochondrial metabolism. Poly P also plays an important role in cancer cell proliferation. The traditional assay for Poly P quantification uses radioisotope methods, which are not convenient to perform. **BioVision's Inorganic Polyphosphate Assay Kit** is a simple assay to quantify the amount of Poly P in various biological samples. The assay uses a fluorescent dye that forms a complex with Poly P present in the samples and the fluorsecent complex is measured at Ex/Em = 415/550 nm. The fluorescent signal is proportional to the Poly P concentration in the samples. BioVision's Inorganic Polyphosphate Assay Kit is rapid, sensitive and a convenient tool for detecting Poly P. It can detect as low as 50 pmole under the assay conditions.

Poly P + Fluorescent Dye ⟶ **Fluorescent Product (Ex/Em = 415/550 nm)**

II. Application:

- Determination of Poly P in different biological samples

III. Sample Type:

- Cell lysate
- Tissue lysate such as kidney, brain, muscle etc.

IV. Kit Contents:

Components	K2025-100	Cap Code	Part Number
Poly P Assay Buffer	100 ml	NM	K2025-100-1
Poly P Extraction Buffer	30 ml	WM	K2025-100-2
Poly P Standard (45 mer)	1 vial	Yellow	K2025-100-3
Poly P Dye	1 vial	Red	K2025-100-4
RNase	400 µl	Blue	K2025-100-5
DNase	400 µl	White	K2025-100-6
Proteinase K	200 µl	Amber	K2025-100-7

V. User Supplied Reagents and Equipment:

- Dounce Tissue Homogenizer (BioVision Cat. # 1998)
- 96-well white flat-bottom plate (BioVision Cat. # M1354)
- Multi-well spectrophotometer
- DMSO

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C. The kit components are stable for one year when stored as recommended. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **Poly P Assay Buffer & Poly P Extraction Buffer:** Ready to use. Warm to room temperature (RT) before use. Store at 4°C.
- **Poly P Standard (45 mer):** Add 1 ml of water to the vial to prepare 100 µM stock Poly P Standard solution. Vortex the tube and let it sit for 5 min at RT. Store at -20°C. Stable for more than 2 months.
- **Poly P Dye:** Add 350 µl of DMSO to the vial. Vortex and let it sit at RT for 5 min. Store at -20°C. Stable for more than 2 months.
- **RNase, DNase and Proteinase K:** Ready to use. Avoid multiple freeze-thaw cycles. Store at -20°C.

VII. Polyphosphate Assay Protocol:

1. Sample Preparation:

Tissue: Transfer ~50 mg of tissue (e.g. kidney, brain and muscle etc.) in an eppendorf tube. Add 250 µl of Poly P Extraction Buffer to the tube and homogenize the tissue for 5 min using dounce tissue homogenizer (BioVision Cat. # 1998). Centrifuge the Sample(s) at 10,000 x *g* for 15 min at 4°C and collect the clear supernatant for the assay.
Bacterial cells: Grow bacteria under the desired experimental conditions. Harvest the bacterial cells by centrifugation. Transfer ~100 mg of cell pellet into an eppendorf tube and add 1 ml of Poly P Assay Buffer to resuspend the cells. Sonicate for 2 min at 4°C on ice and centrifuge at 10,000 x *g* for 15 min at 4°C. Collect the clear cell supernatant. Protein amount in bacterial lysates can be determined by Bradford or BCA assays.

All tested samples from tissue or bacteria require RNase, DNase and Proteinase K treatment. To 100 µl of the Sample(s), add 2 µl of RNase and DNase and incubate for 30-60 min at 37°C. After incubation, take aliquots (e.g. 1-5 µl) to perform agarose gel electrophoresis and check for any residual RNA and DNA. If RNA and/or DNA is detected in the RNase and DNase treated samples, add more nuclease and incubate longer. If no RNA and/or DNA is detected, add 2 µl of Proteinase K to the Sample(s) and incubate at 37°C for 20 min. Heat the samples at 85°C for 10 min and move the samples to an ice bucket. Prepare a well for each Sample to be tested (2-10 µl). Adjust the volume of each well to 50 µl using Poly P Assay Buffer.

2. Standard Curve Preparation: Mix 100 µl of the stock Poly P Standard solution with 900 µl of water to prepare 10 µM Poly P Standard solution. Add 0, 5, 10, 15, 20 and 25 µl of 10 µM Poly P Standard solution into the desired wells to generate 0, 50, 100, 150, 200 and 250 pmole of Poly P Standard/well respectively. Adjust the volume of each well to 50 µl using Poly P Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. Prepare 50 µl of Reaction Mix as indicated below:

	Reaction Mix
Poly P Assay Buffer	47 µl
Poly P Dye	3 µl

Mix well. Add 50 µl of Reaction Mix to all wells including Standards and Samples. Incubate the plate for 10 min at RT.

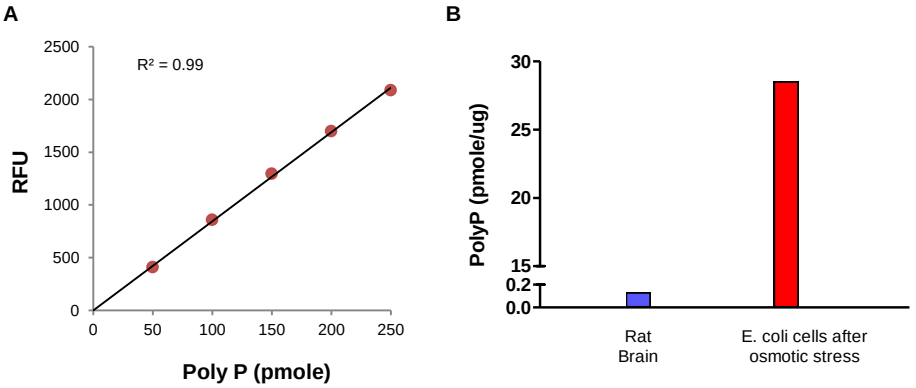
4. Measurement: Measure the fluorescence of all wells at Ex/Em = 415/550 nm at RT in end point mode.

5. Calculation: Subtract the 0 Standard readings from all Standard and Sample readings. Plot the Poly P Standard Curve. Apply the corrected Sample reading to the Poly P Standard Curve to get A pmol of Poly P.

Calculate the total amount of Poly P in the Sample using the following equation:

Amount of Poly P (nmol/mg) = A x D / W

Where: **A** = Amount of Poly P calculated from the Poly P Standard Curve (pmol)
D = Sample dilution factor (D = 1, for undiluted samples)
W = Weight of the tissue used (in mg) or protein amount as determined by the protein assay (mg)



Figures. A. Poly P Standard Curve. **B.** Poly P concentration in rat brain tissue and *E. coli* cells after osmotic stress assayed according to the kit protocol.

VIII. Related Products:

Phosphate Colorimetric Assay Kit (K410)
PicoProbe™ Phosphate Fluorometric Assay Kit (K419)
Pyridoxal 5'-phosphate (Vit B_6) Fluorometric Assay Kit (K2009)
Maltose Phosphorylase Activity Kit (Fluorometric) (K353)
Protein Quantitation Kit (K810)
Phosphate Assay Kit (Fluorometric) (K420)
Pyrophosphate (PPi) Assay Kit (Fluorometric/Colorimetric) (K568)
Phospholipid Assay Kit (Colorimetric/Fluorometric) (K351)
Phospholipase C Activity Assay Kit (Colorimetric) (K2016)
BCA Protein Quantitation Kit (K812)

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