Technical Bulletin

Inorganic Polyphosphate Assay Kit (Fluorometric)

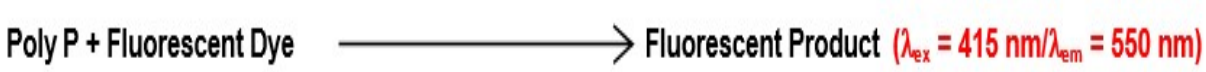
Catalog Number MAK429

Product Description

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 radioisotopic methods, which are not convenient to perform.

The Inorganic Polyphosphate Assay Kit is a simple method 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 sample and the fluorescent complex is measured at λ_{Ex} = 415 nm/λ_{Em} = 550 nm. The fluorescent signal is proportional to the Poly P concentration in the samples. The kit is rapid, sensitive, and is a convenient tool for detecting Poly P. The method can detect as low as 50 pmole under the assay conditions.

The kit is suitable for the determination of Poly P in various biological samples such as cell lysate and tissue (kidney, brain, muscle, etc.) lysate.



Components

The kit is sufficient for 100 fluorometric assays in 96-well plates

- Poly P Assay Buffer 100 mL
  Catalog Number MAK429A
- Poly P Extraction Buffer 30 mL
  Catalog Number MAK429B
- Poly P Standard (45-mer) 1 vial
  Catalog Number MAK429C
- Poly P Dye 1 vial
  Catalog Number MAK429D
- RNase 400 µL
  Catalog Number MAK429E
- DNase 400 µL
  Catalog Number MAK429F
- Proteinase K 200 µL
  Catalog Number MAK429G

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- White flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Dimethyl Sulfoxide (DMSO), anhydrous (Catalog Number 276855 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Bicinchoninic Acid Kit for Protein Determination (Catalog Number BCA1 or equivalent) **OR**
- Bradford Reagent (Catalog Number B6916 or equivalent)
- Agarose gel electrophoresis reagents (agarose gel and running buffer) and equipment (gel box and power supply)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions.

Briefly centrifuge small vials at low speed prior to opening.

Poly P Assay Buffer & Poly P Extraction Buffer: Ready to use. Warm to room temperature prior to use. Store at 2-8 °C.

Poly P Standard (45-mer): Add 1 mL of purified water to the vial to prepare a 100 µM stock Poly P Standard solution. Vortex the tube and let it sit for 5 minutes at room temperature. Store at -20 °C. Stable for more than two months once reconstituted.

Poly P Dye: Reconstitute vial with 350 µL of DMSO. Vortex and let the vial sit at room temperature for 5 minutes. Store at -20 °C. Stable for more than two months once reconstituted.

RNase, DNase and Proteinase K: Ready to use. Avoid multiple freeze-thaw cycles. Store at -20 °C.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Tissue

1. Transfer ~50 mg of tissue (e.g. kidney, brain, muscle, etc.) into an Eppendorf® tube.
2. Add 250 µL of Poly P Extraction Buffer to the tube and homogenize the tissue for 5 minutes using a Dounce tissue homogenizer.
3. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C and collect the clear supernatant for the assay.

Bacterial Cells

1. Grow bacteria under the desired experimental conditions. Harvest the bacterial cells by centrifugation.
2. Transfer ~100 mg of cell pellet into an Eppendorf tube and add 1 mL of Poly P Assay Buffer to resuspend the cells.
3. Sonicate for 2 minutes at 4 °C on ice and centrifuge at 10,000 × *g* for 15 minutes at 4 °C. Collect the clear cell supernatant.

4. Protein amount in bacterial lysates can be determined by Bradford or BCA assays.

Sample Treatment

All tested samples from tissue or bacteria require RNase, DNase and Proteinase K treatment.

1. To 100 µL of the Sample(s), add 2 µL each of RNase and DNase and incubate for 30-60 minutes at 37 °C.
2. After incubation, take aliquots (e.g., 1-5 µL) to perform agarose gel electrophoresis and check for any residual RNA and DNA.
3. If RNA and/or DNA is detected in the RNase and DNase treated samples, add more nuclease and incubate longer.
4. When no RNA and DNA are detected, add 2 µL of Proteinase K to the Sample(s) and incubate at 37 °C for 20 minutes.
5. Heat the Sample(s) at 85 °C for 10 minutes and move the samples to an ice bucket.
6. Prepare a well for each Sample to be tested (2-10 µL). For unknown samples, test several dilutions to ensure that the readings are within the linear range of the Standard Curve.
7. Adjust the total volume of each well to 50 µL using Poly P Assay Buffer.

Standard Curve Preparation

Prepare a 10 µM Poly P Standard solution by diluting 100 µL of the 100 µM Poly P Standard with 900 µL of purified water. Prepare Poly P Standards according to Table 1. Mix well.

Table 1.
Preparation of Poly P Standards

Well	10 µM Poly P Standard	Poly P Assay Buffer	Poly P (pmol/well)
1	0 µL	50 µL	0
2	5 µL	45 µL	50
3	10 µL	40 µL	100
4	15 µL	35 µL	150
5	20 µL	30 µL	200
6	25 µL	25 µL	250

Reaction Mix

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Reaction Mix according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mix

Reagent	Volume
Poly P Assay Buffer	47 µL
Poly P Dye	3 µL

2. Add 50 µL of Reaction Mix to all wells including Standards and Samples. Incubate the plate for 10 minutes at room temperature.

Measurement

Measure the fluorescence of all wells at λ_{Ex} = 415 nm/λ_{Em} = 550 nm at room temperature in end point mode.

Results

1. Subtract the 0 Standard readings from all Standard and Sample readings.
2. Plot the Poly P Standard Curve.
3. Apply the corrected Sample reading to the Poly P Standard Curve to determine A pmol of Poly P.
4. Calculate the total amount of Poly P in the Sample using the following equation:

Amount of Poly P (nmol/mg) =

(A × 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)

Figure 1.
Typical Poly P Standard Curve

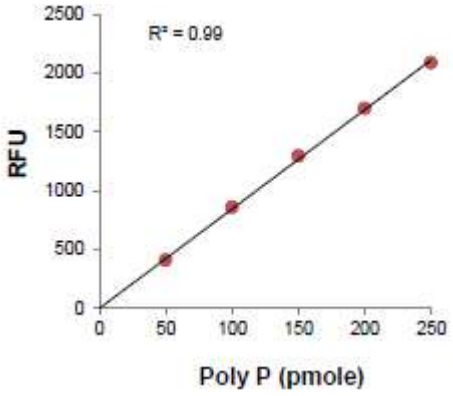
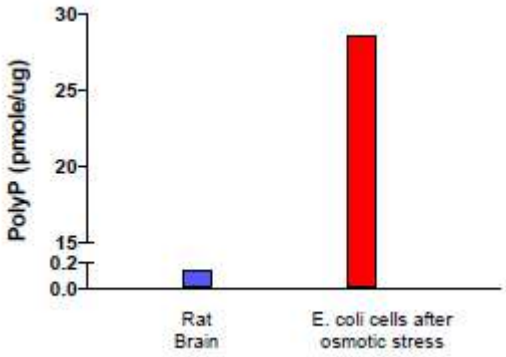


Figure 2.
Poly P concentration in rat brain tissue and *E. coli* cells after osmotic stress. Assayed according to the kit protocol.



Frequently Asked Questions

Is there any limit on the length of the polyphosphate the kit can detect?

There is no maximum limit on the length for the detection. The longer the length, the better the detection as more fluorescent dyes are bound to the sample.

Notice

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