ab284528 – Inorganic Polyphosphate Assay Kit (Fluorometric)

For quantifying the amount of Inorganic Polyphosphate in samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284528

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
DNase	400 µL	-20°C
Polyphosphate Assay Buffer	100 mL	-20°C
Polyphosphate Dye	1 vial	-20°C
Buffer III	30 mL	-20°C
Polyphosphate Standard	1 vial	-20°C
Proteinase K	200 µL	-20°C
RNAse Positive Control	400 µL	-20°C

Materials Required, Not Supplied

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

- Dounce Tissue Homogenizer
- 96-well white flat-bottom plate
- Multi-well spectrophotometer
- DMSO

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Polyphosphate Assay Buffer: Ready to use. Warm to room temperature (RT) before use. Store at 4°C.

Polyphosphate Standard: Add 1 ml of water to the vial to prepare 100 µM stock Polyphosphate Standard. Vortex the tube and let it sit for 5 min at RT. Store at -20°C. Stable for more than 2 months.

Polyphosphate 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 Positive Control: Ready to use. Avoid multiple freeze-thaw cycles. Store at -20°C.

Assay Protocol

Sample Preparation:

Tissue: Transfer ~50 mg of tissue (e.g. kidney, brain and muscle etc.) in an Eppendorf tube. Add 250 µl of Buffer III to the tube and homogenize the tissue for 5 min using dounce tissue homogenizer. 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 Polyphosphate 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.

Δ Note: *All tested samples from tissue or bacteria require RNAse Positive Control, DNase and Proteinase K treatment.*

1. To 100 µl of the Sample(s), add 2 µl of RNAse Positive Control and DNase and incubate for 30-60 min 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 Positive Control and DNase treated samples, add more nuclease and incubate longer.
4. 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.
5. Heat the samples at 85°C for 10 min and move the samples to an ice bucket.
6. Prepare a well for each Sample to be tested (2-10 µl). Adjust the volume of each well to 50 µl using Polyphosphate Assay Buffer.

Standard Curve Preparation:

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

Reaction Mix:

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

Item	Reaction Mix
Polyphosphate Assay Buffer	47 µl
Polyphosphate Dye	3 µl

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

Measurement

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

Calculation

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

Amount of Poly P (pmol/mg) = $A \times D / W \times Vt / Va$

Where: **A** = Amount of Poly P calculated from the Polyphosphate 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)
V_t = Total sample volume
V_a = Volume of sample measured in the well

Technical Support

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