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# Product Information

**Pyrophosphate reagent**

Catalog Number **P7275**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

**Product Description**
Pyrophosphate is determined in this procedure by a series of coupled enzyme reactions. Two moles of NADH are oxidized to NAD per mole of pyrophosphate consumed.

$$PP_i + \text{F-6-P} \xrightarrow{PP_i\text{-PFK}} \text{F-1,6-DP} + P_i$$

$$\text{F-1,6-DP} \xrightarrow{\text{Adolase}} \text{GAP} + \text{DHAP}$$

$$\text{GAP} \xrightarrow{\text{TPI}} \text{DHAP}$$

$$2\ \text{DHAP} + 2\ \beta\text{-NADH} + 2\ H^+ \xrightarrow{\text{GDH}} 2\ \text{glycerol-3-phosphate} + 2\ \beta\text{-NAD}^+$$

The reaction is monitored spectrophotometrically at 340 nm.

Legend

| | |
|---|---|
| $PP_i$ | Pyrophosphate |
| F-6-P | D-Fructose-6-phosphate |
| $PP_i$-PFK | Fructose-6-phosphate kinase, pyrophosphate dependent |
| F-1,6-DP | D-Fructose-1,6-diphosphate |
| $P_i$ | Inorganic phosphate |
| GAP | D-Glyceraldehyde-3-phosphate |
| TPI | Triosephosphate isomerase |
| DHAP | Dihydroxyacetone phosphate |
| GDH | Glycerophosphate dehydrogenase |
| β-NADH | β-Nicotinamide adenine dinucleotide (reduced form) |
| $\beta$-NAD$^+$ | β-Nicotinamide adenine dinucleotide (oxidized form) |

The Pyrophosphate Reagent, Catalog Number P7275, is a lyophilized powder containing coupling enzymes, buffers, salts, and stabilizers. The reagent can be used to detect 10–100 nanomoles of pyrophosphate.

**Reagent Required But Not Provided**
Sodium pyrophosphate decahydrate, Catalog Number 221368

**Precautions and Disclaimer**
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**
Dissolve the contents of one vial of Pyrophosphate Reagent in 4.0 ml of water. After reconstitution, the vial contains the following components at the indicated concentrations:

| Component | Concentration |
|---|---|
| Imidazole · HCl, pH 7.4 | 45 mM |
| Citrate | 5 mM |
| EDTA | 0.10 mM |
| $Mg^{2+}$, $Mn^{2+}$, $Co^{2+}$ | 2 mM, 0.2 mM, 0.02 mM |
| β-NADH | 0.8 mM |
| F-6-P | 12 mM |
| Bovine Serum Albumin | 5 mg/ml |
| Sugar Stabilizer | 5 mg/ml |
| $PP_i$-PFK | 0.5 units/ml |
| Aldolase | 7.5 units/ml |
| GDH | 5 units/ml |
| TPI | 50 units/ml |

**Storage/Stability**
Store the Pyrophosphate Reagent at –20 °C.

The reconstituted reagent remains active up to 24 hours when store at 2–8 °C.

**Procedure**

1. Reconstitute Pyrophosphate Reagent, see Preparation Instructions.
   Note: Do not dilute the Pyrophosphate Reagent to lower the initial $A_{340}$ of the reaction mixture, since this will dilute the components below kinetically effective concentrations.

2. Prepare a 1.0 mM Pyrophosphate Standard by dissolving 44.61 mg of sodium pyrophosphate decahydrate, Catalog Number 221368, in 100 ml of water.

3. Sample to be tested should contain 200–2000 nanomoles of pyrophosphate per ml.

4. To 3 ml cuvettes (1 cm light path), add:

| Reagent | Blank (ml) | Test (ml) | Standard (ml) | Control (ml) |
|---|---|---|---|---|
| Reconstituted Pyrophosphate Reagent | 1.00 | 1.00 | 1.00 | 1.00 |
| Water | 1.95 | 1.95 | 1.95 | 1.95 |

5. Mix by inversion. The initial $A_{340}$ of the reaction mixture should be ~1.5. Equilibrate to 30 °C using a thermostated spectrophotometer. Record the initial $A_{340}$ vs. water for each cuvette, then add:

| Reagent | Blank (ml) | Test (ml) | Standard (ml) | Control (ml) |
|---|---|---|---|---|
| Pyrophosphate Standard | – | – | 0.05 | 0.05 |
| Sample | – | 0.05 | – | 0.05 |
| Water | 0.05 | – | – | – |

6. Mix by inversion and monitor decrease in $A_{340}$ at 30 °C for all cuvettes until no further decrease is observed (~10 minutes). Record the final $A_{340}$ for each cuvette.

**Results**

Calculations

$\Delta A_{TEST}$ = Initial $A_{340}$ Test – Final $A_{340}$ Test

$\Delta A_{BLANK}$ = Initial $A_{340}$ Blank – Final $A_{340}$ Blank

Pyrophosphate, micromoles/ml in sample =

$$\frac{\Delta A_{TEST} - \Delta A_{BLANK} \times 3}{6.22 \times 2 \times 0.05} =$$

$$(\Delta A_{TEST} - \Delta A_{BLANK}) \times 4.82$$

3 = Volume of reaction mixture
6.22 = Millimolar absorbtivity of NADH at 340 nm
2 = moles of β-NADH oxidized per mole of pyrophosphate consumed
0.05 = Volume of sample

Notes: Standard – 95–100% recovery of 50 nanomoles of pyrophosphate will result in a $\Delta A_{340}$ of ~0.2.

$\Delta A_{CONTROL}$ should equal 0.2 + $\Delta A_{SAMPLE}$

**References**

1. O'Brien, W., Anal. Biochem., **76**, 423 (1976).

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