

Phosphate Assay for DMADP Quantification

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Materials:

Inorganic Pyrophosphatase (Sigma I1653)
DMADP
KH₂PO₄ Standards 5, 3, 2, 1, 0.5, mM
20 mM Ammonium Molybdate
1.6 M HCl
1 U/μl inorganic pyrophosphatase
50 mM MgCl₂
100 mM Ascorbic Acid
Spectrophotometer and plates

Procedure:

1. Incubate 10 μl of 2 mM DMADP with 2 μL of Inorganic Phosphatase and 3 μL 50 mM MgCl₂ on the bench for one hour in a 96-well plate.
2. Make the following Molybdate/HCl mix per sample being tested
+ 70 μl 20 mM ammonium molybdate
+ 70 μl 1.6 M HCl.
Remember to included your standards when you decide how many samples you will run, also it is best to do standards and samples in triplicate.
3. Add 140 μL of the HCl/Ammonium Molybdate mixture to each well.
4. Add 60 μl 100 mM Ascorbic Acid to each well.
5. Incubate on the bench for 20 minutes.
6. Read at 690 nm on the spectrophotometer

Reagents:

Note: To avoid Pi contamination prepare all reagents in 50 ml disposable falcon tubes using disposable pipettes/tips. It is best to avoid use of glassware or any other washed materials.

200 mM NH₄HCO₃, 50 ml

In 50 ml dH₂O
+ 0.79 g NH₄HCO₃ (FW 79.06)
pH to 7.0 and store at 4°C (you may filter sterilize)

2 mM NH₄HCO₃, 50 ml

In 49.5 mL dH₂O
+ 500 μl 200 mM NH₄HCO₃
pH to 7.0 and store at 4°C (you may filter sterilize)

≈ 2 mM DMADP, 1.68 ml

Take 1 mg of DMADP and add 1.68 ml 2 mM NH₄HCO₃
Aliquot into 200 μl aliquots and store in -80°C

1 U / μl Inorganic Pyrophosphatase, 500 μl

To 500 units of Sigma I1643 add 500 μl 150 mM Hepes Buffer pH 7.2 with 30% glycerol

Divide into 100 μ l aliquots and store in -80°C

20 mM Ammonium Molybdate, 50 mL

Do this in the fume hood

In 50 ml of dH_2O

+ 1.25 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (FW 1235.86)

Adjust volume to 250 ml

1.6 M HCl, 50 ml

In 40 ml of dH_2O

+ 6.6 ml of 12.1 M ($\approx 37\%$) HCl

**** Remember acid into water never water into acid****

Adjust volume to 50 ml

100 mM Ascorbic Acid, 15 ml

In 15 ml dH_2O

+ 0.26 g Ascorbic Acid (FW 176.12)

Aliquot into 2 ml tubes and store in -80°C

KH_2PO_4 Standards

Dry some KH_2PO_4 powder overnight at 200°C

In 10 ml 2 mM NH_4HCO_3

+ 0.027 g KH_2PO_4 20 mM

Standard KH_2PO_4 (mM)	2 mM NH_4HCO_3 (ml)	20 mM KH_2PO_4 (μ l)
5	1.5 ml	500 μ l
3	1.7 ml	300 μ l
2	1.8 ml	200 μ l
1	1.9 ml	100 μ l
0.5	1.95 ml	50 μ l
0.3	1.97 ml	30 μ l
0.2	1.98 ml	20 μ l
0.1	1.99 ml	10 μ l
0.05	1.995 ml	5 μ l