Phosphate Assay for DMADP Quantification

Materials:
- Inorganic Pyrophosphatase (Sigma I1653)
- DMADP
- KH$_2$PO$_4$ Standards 5, 3, 2, 1, 0.5, mM
- 20 mM Ammonium Molybdate
- 1.6 M HCl
- 1 U/µl inorganic pyrophosphatase
- 50 mM MgCl$_2$
- 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 MgCl$_2$ 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 NH$_4$HCO$_3$, 50 ml
In 50 ml dH$_2$O
+ 0.79 g NH$_4$HCO$_3$ (FW 79.06)
pH to 7.0 and store at 4°C (you may filter sterilize)

2 mM NH$_4$HCO$_3$, 50 ml
In 49.5 mL dH$_2$O
+ 500 µl 200 mM NH$_4$HCO$_3$
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 NH$_4$HCO$_3$
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 µl aliquots and store in -80°C

**20 mM Ammonium Molybdate, 50 mL**
Do this in the fume hood
In 50 ml of dH₂O
+ 1.25 g (NH₄)₆Mo₇O₂₄ * 4H₂O (FW 1235.86)
Adjust volume to 250 ml

**1.6 M HCl, 50 ml**
In 40 ml of dH₂O
+ 6.6 ml of 12.1 M (≈37%) HCl
**Remember acid into water never water into acid**
Adjust volume to 50 ml

**100 mM Ascorbic Acid, 15 ml**
In 15 ml dH₂O
+ 0.26 g Ascorbic Acid (FW 176.12)
Aliquot into 2 ml tubes and store in -80°C

**KH₂PO₄ Standards**
Dry some KH₂PO₄ powder overnight at 200°C
In 10 ml 2 mM NH₄HCO₃
+ 0.027 g KH₂PO₄ 20 mM

<table>
<thead>
<tr>
<th>Standard KH₂PO₄ (mM)</th>
<th>2 mM NH₄HCO₃ (ml)</th>
<th>20 mM KH₂PO₄ (µl)</th>
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<tbody>
<tr>
<td>5</td>
<td>1.5 ml</td>
<td>500 µl</td>
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<tr>
<td>3</td>
<td>1.7 ml</td>
<td>300 µl</td>
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<td>2</td>
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<td>1</td>
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<tr>
<td>0.05</td>
<td>1.995 ml</td>
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