

Preparation of NMR Buffers Standard Operating Procedure

SOP # 005.1 Version # 1

1. Scope:

This document applies to the preparation procedures of Sodium phosphate buffers used in the dissolution of solid compound samples (NMR Buffers) for the Human Metabolome Project.

2. Objective:

This protocol describes the steps for the preparation of Sodium phosphate buffers (NMR Buffer).

3. Preparation of NMR Buffers Protocol:

3.1. Stock Solutions:

3.1.1. 1M stock solution of Na_2HPO_4 .

Weigh 141.96g of Na_2HPO_4 (anhydrous) and dissolve to 1000 ml (one Liter) with ddH₂O.

3.1.2. 1M stock solution of NaH_2PO_4 .

Weigh 119.98g of NaH_2PO_4 (anhydrous) and dissolve to 1000 ml (one Liter) with ddH₂O.

NB: The weight of each salt needed to prepare one liter of a 1M solution varies, depending on the hydrated state of the salt.

3.1.3. 50mM stock solution of DSS.

Weigh 1.0915g of DSS and dissolve to 100 ml with ddH₂O.

3.1.4. NaOH solution.

3.1.5. HCl solution.

3.2. NMR Buffer number 1:

3.2.1. Measure 28.85 ml of the 1M stock solution of Na_2HPO_4 and 21.15 ml of the 1M stock solution of NaH_2PO_4 into a 1000 ml graduated cylinder.

3.2.2. Mix and add ddH₂O to 850 ml.

3.2.3. Add 100 ml of D₂O.

3.2.4. Add 10 ml of the 50 mM DSS stock solution.

3.2.5. Mix well and adjust to pH to 7.0 (if necessary) with HCL or NaOH solution.

3.2.6. Make volume up to 1000 ml with more ddH₂O

3.2.7. Store in a labeled, dated bottle in the refrigerator.

3.2.8. Check for impurities before use.

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3.3.NMR Buffer number 2:

3.3.1.Measure 28.85 ml of the 1M stock solution of Na_2HPO_4 and 21.15 ml of the 1M stock solution of NaH_2PO_4 into a 1000 ml graduated cylinder.

3.3.2.Mix and add ddH₂O to 980 ml.

3.3.3.Mix well and adjust to pH to 7.0 (if necessary) with HCL or NaOH solution.

3.3.4.Make volume up to 1000 ml with more ddH₂O

3.3.5.Store in a labeled, dated bottle in the refrigerator.

3.3.6.Check for impurities before use.

4. Materials.

4.1. Na_2HPO_4

4.2. NaH_2PO_4

4.3.DSS

4.4.D₂O

4.5.ddH₂O

4.6.NaOH

4.7.HCl

4.8.Weighing material

5. Equipment.

5.1.Digital balance

5.2.pH meter

5.3.Graduated 100 ml cylinder

5.4.Graduated 1000 ml cylinder

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6. Documentation.

6.1.MSDS

6.2.Adapted from: Molecular Cloning: A laboratory manual, Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) 2nd Edition, Cold Spring Harbour Laboratory Press, Book 3 Appendix B2.

7. Safety.

See MSDS.

8. Records.

N/A

9. Responsibilities.

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