Version 2

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Preparation of PBS Solution V.2

In 1 collection

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dx.doi.org/10.17504/protocols.io.3byl4bxjrvo5/v2

Low-cost, high-quality ...

Nadine Mowoh

ABSTRACT

Phosphate buffered Saline (PBS) is a buffer solution commonly used in biological research. It is a salty solution containing sodium chloride, sodium phosphate, and (in some formulations) potassium chloride and potassium phosphate. The buffer helps to maintain a constant pH. This SOP gives one of the most common formulations used in preparing PBS (1x PBS).

- 137 mM NaCl
- 10 mM Na2HPO4
- 1.8 mM KH2PO4
- pH 7.2 -7.4

PBS can be used as a diluent in methods to dry biomolecules, as water molecules within it will be structured around the substance (protein, for example) to be 'dried' and immobilized to a solid surface. It is applied in washing cells after protein expression procedures due to its osmolarity and pH to avoid cell disruption to preserve and subsequently freeze the cells for downstream molecular biology applications.

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PROTOCOL CITATION

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COLLECTIONS

 **Beneficial Bio: Internal protocols**

KEYWORDS

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PARENT PROTOCOLS

Part of collection
Beneficial Bio: Internal protocols

GUIDELINES

This protocol can be performed by anyone with basic lab skills

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MATERIALS TEXT

Reagents

- Sodium chloride **Sigma – Aldrich**
- Sodium phosphate dibasic heptahydrate **Acros Organics**
- Potassium phosphate monobasic **Acros Organics**
- Sodium hydroxide **Contributed by users**
- Hydrochloric acid **Fisher Scientific** Step 3

Materials and equipment

- 500 mL Beaker
- 100 mL Measuring Cylinder
- Distilled water
- pH Meter
- Electronic Balance
- Weighing boat
- Magnetic Stirrer
- Refrigerator
- Autoclave
- 1000 mL Duran Bottle

SAFETY WARNINGS

- Always put on the right Personal Protective Equipment to minimize or eliminate chances of accidental spill or splashes on the body or in the eye.
- Special caution should be taken while handling HCL because it it corrosive

BEFORE STARTING

Make sure all salts and equipment to be used are available in the right quantities and functional respectively.

Preparing reagents and workspace

1 10m

- Before beginning the procedure, put on Personal Protective Equipment (Lab Gown, Gloves, shoes, masks and goggles).
- Clean up work surfaces and environment using disinfection solution (Bleach and after 70% alcohol).
- Ensure that all reagents and equipment needed for the procedure are available, in place and functional.

Preparing 1 M NaOH

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- Weigh 19.95 g of NaOH pellets & dissolve them in half liter (500 mL) of distilled water water.

Preparing 3 M HCL

- Measure out 8.5 mL of distilled water into a cleaned and dried 50 mL volumetric flask. Add 2.5 mL of Concentrated HCL.

> Remember to add acid to water, the volumetric flask should be part filled with water before adding the acid.

Weighing and dissolving salts 6m

2 3m

Use an electronic balance to accurately weigh out all salt powders
Sodium chloride **Sigma – Aldrich** ,
Potassium phosphate monobasic **Acros Organics** and
Sodium phosphate dibasic heptahydrate **Acros Organics** into an appropriate size weighing boat with the help of a spatula as indicated in the table below.

Composition of 1X PBS buffer

A	B	C	D
Salt	**Concentration (mmol/L)**	**Concentration (g/L)**	**Amount in grams (g) for 500 mL**
NaCl	137	8.0	4.0
Na2HPO4	10	1.42	0.71
KH2PO4	1.8	0.24	0.12

- Transfer the contents of the weighing boat into a 500 mL or larger beaker.
- Use a 100ml measuring cylinder to measure out 100 mL of sterile distilled water and pour carefully into the beaker and rinse the boat to take out all the powder residue.
- Mix gently by swirling. Use a magnetic flea to stir the solution for **00:03:00** on a magnetic stirrer to rinse the boat to take out any powder residue' completely dissolve the salts.

3 5m

Check the pH of the solution and either add 1M

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Sodium hydroxide **Contributed by users Catalog #795429** or 3 M Hydrochloric acid **Fisher Scientific** gradually to adjust the to pH **7.2** or pH **7.4** as desired.

- After confirming the pH, measure out the appropriate amount of sterile distilled water to make up the volume of the solution to 500 mL of 1x PBS.

Sterilisation and storage

4 Transfer the contents of the beaker into a 1000 mL Duran bottle and autoclave to make the PBS solution sterile. 30m

5 Remove the 1x PBS from the autoclave, allow it to cool, cork tightly and store at **Room temperature** or in the refrigerator and store at **4 °C** . The 1x PBS buffer is ready for use in any molecular biology technique.

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