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Preparation of PBS Solution

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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.

Stephane Fadanka, Shalo Minette, Nadine Mowoh 2022. Preparation of PBS Solution. **protocols.io**
https://protocols.io/view/preparation-of-pbs-solution-b85dry26

PBS, Phosphate Buffered Saline

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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

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

Always put on the right Personal Protective Equipment to minimise or eliminate chances of accidental spill or splashes on the body or in the eye.

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

Preparing reagents and workspace 5m

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- 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).
- Check to be sure all reagents and equipment needed for the procedure are available, in place and functional.

Weighing and dissolving salts 6m

3m

2 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 into an appropriate size weighing boat with the help of a spatula in recommended amounts as indicated in the table below.

Composition of 1X PBS buffer

A	B	C	D
Salt	**Concentration (mmol/L)**	**Concentration (g/L)**	**Amount 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 500ml or larger beaker.
- Use a 100ml measuring cylinder to measure 100ml of sterile distilled water and pour carefully to rinse the boat so as to take out all the powder into the beaker.
- Mix gently by swirling and take the beaker onto a magnetic stirrer and put the magnetic flea for about **00:03:00** to completely dissolve the salts.

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Use a pH meter to measure the pH of the solution and either add

Sodium hydroxide **Contributed by users Catalog #795429** or

Hydrochloric acid **Fisher Scientific** to adjust the to pH **7.2** or pH **7.4** as desired.

- Measure out the appropriate amount of sterile distilled water to make up the volume of the solution to 500ml of 1x PBS.

Sterilisation and storage 20m

4 Transfer the contents of the beaker into a 500ml or 1000ml Duran Bottle and autoclave to make the PBS solution sterile.

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.