

User Guide: Buffer Formulations

Buffers from Scratch

Instructions

Prepare buffers fresh each day. Storing user-prepared 1X buffers for extended periods comes with risks such as precipitation, growth, or contamination which can have an effect on test performance. For those willing to assume this risk Agdia has recommended storage conditions and preservatives where possible.

Chemicals from different vendors can vary in purity level and lot-to-lot consistency, which can affect test results. You assume this risk when purchasing chemicals separately to make buffers per these formulations.

Pre-mixed buffer packs may also be purchased from Agdia. Buffer packs are recommended since Agdia evaluates suppliers to reduce the risk of inconsistent test performance attributed to variation in raw material quality.

Safety

Agdia recommends reading all relevant SDS sheets.

Carbonate Coating Buffer (CCB) (1X) 1. Used to dilute capture antibodies. 2. Dissolve components in 800 mL of water ¹ . 3. Adjust pH to the range of 9.5 - 9.7. 4. Adjust volume to 1000 mL with water ¹ . 5. Optional: Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.	Sodium carbonate (anhydrous) 1.59 g Sodium bicarbonate 2.93 g Water ¹ 1000 mL
General Extract Buffer (GEB) (1X) 1. GEB is used to grind and dilute samples. 2. Dissolve components in 800 mL of 1X PBST. 3. Adjust pH to the range of 7.2 - 7.8. 4. Adjust volume to 1000 mL with 1X PBST. 5. Optional: Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.	Sodium sulfite (anhydrous) 1.30 g Polyvinylpyrrolidone (PVP) MW 24-40,000 20.00 g Powdered egg (chicken) albumin, Grade II 2.00 g TWEEN® 20 20.00 g 1X PBST 1000 mL
ECI Buffer (1X) 1. ECI is used to dilute enzyme conjugate antibodies. 2. Dissolve components in 800 mL of 1X PBST. 3. Adjust pH to the range of 7.2 - 7.6. 4. Adjust volume to 1000 mL with 1X PBST. 5. Optional: Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.	Bovine serum albumin (BSA) 2.00 g Polyvinylpyrrolidone (PVP) MW 24-40,000 20.00 g 1X PBST 1000 mL
PNP Substrate Buffer (1X) 1. PNP substrate buffer is used with PNP substrate tablets to make an active substrate for alkaline phosphatase ELISA systems. 2. Dissolve components in 800 mL of water ¹ . 3. Adjust pH to the range of 9.7 - 9.9 with hydrochloric acid. 4. Adjust volume to 1000 mL with water ¹ . 5. Optional: Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.	Magnesium chloride hexahydrate 0.10 g Diethanolamine 97.00 mL Water ¹ 1000 mL

¹Use deionized or similar purity water.



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PBST Buffer (Wash Buffer) (1X) 1. PBST buffer is used to wash ELISA plates. 2. Dissolve components in 800 mL of water ¹ . 3. Adjust pH to the range of 7.2 - 7.6. 4. Adjust volume to 1000 mL with water ¹ . 5. Optional: Store at 18 - 30 °C. Sodium azide is not recommended.	Sodium chloride 8.00 g Sodium phosphate, dibasic (anhydrous) 1.15 g Potassium phosphate, monobasic (anhydrous) 0.20 g Potassium chloride 0.20 g TWEEN® 20 0.50 g Water ¹ 1000 mL
PBST Buffer (Stock Solution) (20X) 1. 20X PBST buffer, Stock Solution, can be stored and diluted to 1X PBST as needed. 2. Dissolve components in 800 mL of water ¹ . 3. Adjust volume to 1000 mL with water ¹ . 4. Store at 18 - 30 °C. Sodium azide is not recommended. Shelf life is one year. Preparing 1X PBST from 20X PBST 5. Dilute 1 volume of 20X PBST concentrate with 19 volumes of deionized or similar purity water before use. <i>Example: To prepare 1000 mL of 1X PBST, mix 50 mL of 20X PBST concentrate with 950 mL of water.</i> 6. Adjust the pH to the range of 7.2 to 7.6. 7. Optional: Store at 18 - 30 °C. Sodium azide is not recommended.	Sodium chloride 160.00 g Sodium phosphate, dibasic (anhydrous) 23.00 g Potassium phosphate, monobasic (anhydrous) 4.00 g Potassium chloride 4.00 g TWEEN® 20 10.00 g Water ¹ 1000 mL
PBS Buffer (1X) 1. PBS Buffer is used as a solvent in MPBS. 2. Dissolve components in 930 mL of water ¹ . 3. Adjust pH to the range of 7.3 - 7.5. 4. Adjust volume to 1000 mL with water ¹ . 5. Optional: Store at 18 - 30 °C. Sodium azide is not recommended.	Sodium phosphate, dibasic (anhydrous) 1.15 g Potassium chloride 0.20 g Potassium phosphate, monobasic (anhydrous) 0.20 g Sodium chloride 8.00 g Water ¹ 1000 mL
Indirect Sample Extraction Buffer (IEB) (1X) 1. IEB is used to grind and dilute samples. 2. Dissolve components in 800 mL of water ¹ . 3. Adjust pH to the range of 9.5 - 9.7. 4. Adjust volume to 1000 mL with water ¹ . 5. Optional: Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.	Sodium carbonate (anhydrous) 1.59 g Sodium bicarbonate 2.93 g Polyvinylpyrrolidone (PVP) MW 24-40,000 20.0 g Water ¹ 1000 mL
MEB Buffer (1X) 1. MEB is used to grind and dilute samples. 2. Dissolve components in 200 mL of 1X PBST. 3. Stir for 30 minutes. 4. Adjust pH to the range of 7.2 - 7.8. 5. Optional: Store at 2 - 8 °C. Sodium azide is not recommended.	TWEEN® 20 1.25 g Nonfat dried milk 1.00 g 1X PBST 200 mL

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ECM Buffer (1X) 1. ECM is used to dilute enzyme conjugate antibodies. 2. Dissolve components in 100 mL of 1X PBST. 3. Stir for 30 minutes. 4. Adjust pH to the range of 7.2 - 7.6. 5. Optional: Store at 2 - 8 °C. Sodium azide is not recommended.	Nonfat dried milk 0.40 g 1X PBST 100 mL
MPBS Buffer (1X) (BRA Blocking Buffer) 1. MPBS is used to block Bacterial Reagent Set (BRA) ELISA plates. 2. Dissolve components in 18 mL of 1X PBS. 3. Stir for 30 minutes. 4. Adjust pH to the range of 7.2 - 7.6. 5. Adjust volume to 20 mL with 1X PBS. 6. Optional: Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.	Nonfat dried milk 1.00 g 1X PBS 20 mL
MPBST Buffer (1X) (BRA Antibody Diluent) 1. MPBST is used to dilute enzyme conjugate antibodies. 2. Dissolve components in 20 mL of 1X PBST. 3. Stir for 30 minutes. 4. Adjust pH to the range of 7.2 - 7.6. 5. Optional: Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.	Nonfat dried milk 0.50 g 1X PBST 20 mL

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