

APPENDIX M
OHIO WATER MICROBIOLOGY LABORATORY
BUFFER PREPARATION

Phosphate Buffer with Magnesium Chloride preparation

STOCK SOLUTIONS:

Stock KH_2PO_4 solution

1. Dissolve 34.0 g of KH_2PO_4 (anhydrous potassium dihydrogen phosphate) in 500 mL deionized water. Adjust to pH 7.2 with 1 N NaOH.
2. Bring volume to 1 L using deionized water.
3. Autoclave at 121°C for 15 minutes.

Stock MgCl_2 solution

1. Dissolve 38 g anhydrous MgCl_2 (or 81.1g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) in 1 L deionized water.
2. Autoclave at 121°C for 15 minutes.

Store sterilized stock solutions at room temperature for up to 1 year until opened. Once open, store at 4°C. If buffer becomes cloudy and there is evidence of contamination, discard the buffer and prepare a fresh solution.

Clearly mark that this is concentrated stock solution and NOT working solution.

WORKING SOLUTION:

1. Mix 1.25 mL stock KH_2PO_4 solution and 5 mL stock MgCl_2 solution per 1 L of deionized water.
2. Dispense in appropriate amounts that will provide either 99 mL in dilution bottles or a larger volume for use as rinse water (containers should be no more than $\frac{3}{4}$ full).
3. Loosen lids and autoclave at 121°C for at least 15 minutes. (Larger volumes need longer autoclave times). Tighten lids when buffer has cooled.

Store sterile buffer at room temperature for up to 1 year until opened. Once open, store at 4°C for up to 3 days. If buffer becomes cloudy and there is evidence of contamination, discard the buffer and prepare a fresh solution.