

Phosphate Buffer, pH 7.2

Intended Use

Phosphate Buffer, pH 7.2 is used for the preparation of dilution blanks for use in the examination of waters, dairy products, foods and other materials.

Meets United States Pharmacopoeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP)¹⁻³ performance specifications, where applicable.

User Quality Control

Identity Specifications

BBL™ Phosphate Buffer, pH 7.2

Dehydrated Appearance: White, fine, homogeneous, free of extraneous material.

Solution: 3.4% solution, soluble in purified water. Solution is colorless, clear to trace hazy.

Prepared Appearance: Colorless, clear to trace hazy.

Stock Solution at 25°C: pH 7.2 ± 0.5

BBL™ Phosphate Buffer, pH 7.2 (prepared)

Appearance: Colorless to light yellow and clear to trace hazy.

Reaction at 25°C: pH 7.2 ± 0.1

Survival Test

BBL™ Phosphate Buffer, pH 7.2 (prepared, 500 mL bottle, Working Solution)

Perform a 10-minute survival test on the buffer using appropriate dilutions of organisms. In a sterile Petri dish, combine 1 mL of organism dilution with 20 mL of autoclaved and cooled (45-50°C) Standard Methods Agar or Tryptic Soy Agar. Incubate at 35 ± 2°C for up to 3 days.

ORGANISM	APCC ⁴	INOCULUM CFU	RECOVERY AT 10 MINUTES
<i>Bacillus cereus</i>	11778	30-300	≥85% of time zero counts
<i>Candida albicans</i>	10231	30-300	≥85% of time zero counts

BBL™ Phosphate Buffer, pH 7.2 (prepared, 100 mL bottle, Stock Solution)

Perform a 10-minute survival test on a 1:800 dilution of the stock solution using appropriate dilutions of organisms and the membrane filtration test. Incubate organisms for up to 5 days at 30-35°C. Incubate *A. brasiliensis* and *C. albicans* at 20-25°C for up to 5 days.

ORGANISM	APCC ⁴	INOCULUM CFU	RECOVERY AT 10 MINUTES
<i>Aspergillus brasiliensis</i> (spore)	16404	10-100	>80% of time zero counts
<i>Bacillus subtilis</i>	6633	10-100	>80% of time zero counts
<i>Candida albicans</i>	10231	10-100	>80% of time zero counts
<i>Escherichia coli</i>	8739	10-100	>80% of time zero counts
<i>Pseudomonas aeruginosa</i>	9037	10-100	>80% of time zero counts
<i>Salmonella Albany</i> DSM 4224		10-100	>80% of time zero counts
<i>Staphylococcus aureus</i>	6538	10-100	>80% of time zero counts

Summary and Explanation

The formula for phosphate buffer was originally specified by the American Public Health Association (APHA) for use in diluting test samples. Phosphate Buffer, pH 7.2 still is specified for use in diluting water samples,⁴ dairy products⁵ and foods⁶⁻⁷ in standard microbiological methods. In some compendial methods,^{8,9} this product is referred to as Butterfield's Phosphate Buffered Dilution Water. Some methods⁴ require the addition of 5.0 mL of a magnesium chloride solution (81.1 g MgCl₂ • 6H₂O per L of purified water) to the product. General chapters <61> and <62> of the USP recommend the use of Phosphate Buffer, pH 7.2 for preparing dilutions of nonsterile pharmaceutical products when performing Microbial Enumeration Tests and Tests for Specified Microorganisms.¹

Principles of the Procedure

Phosphate buffer is used in the preparation of dilution blanks for use in microbiological testing rather than unbuffered water in order to standardize this potential variable due to the wide variation in the pH of purified water from multiple sources. Sodium carbonate is a pH regulator.

Formula

BBL™ Phosphate Buffer, pH 7.2

Approximate Formula* Per Liter	
Potassium Dihydrogen Phosphate	26.22 g
Sodium Carbonate	7.78 g

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Prepare a stock solution, according to standard procedure, by dissolving 34.0 g in purified water and make up to 1 L.
2. Dispense and sterilize, if desired. Store under refrigeration.
3. Prepare a working solution for use in dilution blanks, according to standard procedure, by adding 1.25 mL of stock solution to purified water⁴ and make up to 1 L (1:800).
4. Dispense in bottles or tubes to provide a post-autoclaving volume of 99 ± 2 mL or 9 ± 0.2 mL or other appropriate quantity.
5. Autoclave at 121°C for 15 minutes.

*NOTE: If desired, add 5.0 mL of magnesium chloride solution (81.1 g MgCl₂ • 6 H₂O per L of purified water).

Sample Collection and Handling

For water, dairy and food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.^{4,5,6}

For pharmaceutical samples, refer to the USP for details on sample collection and preparation for testing of nonsterile products.¹

Phosphate Buffer Ph 7 Becton Dickinson

Michael Rajnik



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