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 Pharmacopeia (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	ATCC™	INOCULUM CFU	RECOVERY AT 10 MINUTES
Bacillus cereus	11778	30-300	≥85% of time zero counts
Candida albicans	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	ATCC™	INOCULUM CFU	RECOVERY AT 10 MINUTES
Aspergillus brasiliensis (niger)	16404	10-100	>80% of time zero counts
Bacillus subtilis	6633	10-100	>80% of time zero counts
Candida albicans	10231	10-100	>80% of time zero counts
Escherichia coli	8739	10-100	>80% of time zero counts
Pseudomonas aeruginosa	9027	10-100	>80% of time zero counts
Salmonella Abony DSM 4224		10-100	>80% of time zero counts
Staphylococcus aureus	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 products[5] and foods[6-9] in standard microbiological methods. In some compendial methods,[6,7] this product is referred to as Butterfield's Phosphate Buffered Dilution Water. Some methods[4] require the addition of 5.0 mL of a magnesium chloride solution (81.1 g $MgCL_2 \bullet 6H_2O$ 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 water* 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 $MgCl_2 \bullet 6 H_2O$ 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.[4-9]

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

Procedure

For water, dairy and food samples, refer to appropriate standard references for details on test methods for using Phosphate Buffer, pH 7.2.[4-9]

For pharmaceutical samples, refer to *USP* General Chapters <61> and <62> for details on the examination of nonsterile products using Phosphate Buffer, pH 7.2.[1]

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.
5. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. U.S. Food and Drug Administration. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
8. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
9. U.S. Department of Agriculture. Microbiology laboratory guidebook, online. Food Safety and Inspection Service, USDA, Washington, D.C.

Availability

BBL™ Phosphate Buffer, pH 7.2

AOAC BAM COMPF EP JP SMD SMWW USDA USP

Cat. No.	211544	Dehydrated – 500 g†
	214973	Prepared Bottles (Working Solution), 500 mL (septum screw cap) – Pkg. of 10†
	257385	Prepared Bottles (Stock Solution), 100 mL (septum screw cap) – Ctn. of 25†

† QC testing performed according to USP/EP/JP performance specifications.