

## Buffers



Microparticle buffers should be suitable for both the ligand and the task at hand. A generic biologic buffer such as PBS or PBS-Tween® may be utilized for bead washes prior to coating, while more specific / complex buffers will typically be used for microparticle coating, storage and use. See PDS 738 for our range of prepared microsphere buffers and suspending solutions (BUFF1 – BUFF6, SOLN1).

### PURPOSE BUFFER CONSIDERATIONS

Pre-washes	Remove residuals Normalize environment Should not contain additives that will compete with ligand for the bead surface
Coating	Provides conditions needed for the reaction (such as pH for EDAC activation of COOH groups) Provides conditions that are optimal for the ligand (pH, concentration, etc.) Potential inclusion of blocker
Storage	Provides conditions that are optimal for the ligand (pH, concentration, etc.) Inclusion of additives that promote stability, e.g. blocker, detergent, antimicrobial agent
Assay	Composition promotes high specific binding, low nonspecific binding for the specific assay system

Following are some basic recipes for buffers commonly used in microsphere coating.

#### Citrate-Phosphate Buffer; pH range 2.6 to 7.0

0.1 M Citric acid: 19.2 g/L (MW 192.1)  
0.2 M Dibasic sodium phosphate: 35.6 g/L (dihydrate; MW 178.0)

Mix citric acid and sodium phosphate solutions in the proportions indicated and adjust the final volume to 100mL with deionized water. Adjust the final pH using 1N HCl or 1 N NaOH.

mL Citric Acid	44.6	35.9	29.4	24.3	19.7	13.6	6.5
mL of Na Phosphate	5.4	14.1	20.6	25.7	30.3	36.4	43.6
pH	2.6	3.4	4.2	5.0	5.8	6.6	7.0

#### Acetate Buffer; pH range 3.6 to 5.6

0.1 M Acetic acid: (5.8mL made to 1000mL)  
0.1 M Sodium acetate: 8.2 g/L (anhydrous; MW 82.0)

Mix acetic acid and sodium acetate solutions in the proportions indicated and adjust the final volume to 100mL with deionized water. Adjust the final pH using 1N HCl or 1 N NaOH.

mL Acetic Acid	46.3	41.0	30.5	20.0	14.8	10.5	4.8
mL of Na Acetate	3.7	9.0	19.5	30.0	35.2	39.5	45.2
pH	3.6	4.0	4.4	4.8	5.0	5.2	5.6

#### MES Buffer; pH range 5.7 to 7.2

Dissolve 19.2g of MES free acid (MW 195.2) in ~900mL of pure water. Titrate to desired pH with 1 N HCl or 1 N NaOH and adjust final volume to 1000mL with pure water.

**Phosphate Buffered Saline (PBS); pH 7.4**

Potassium Phosphate dibasic: 1.82 g/L (MW 174.2)

Sodium Phosphate monobasic: 0.22 g/L (MW 120.0)

Sodium Chloride: 8.76 g/L (MW 58.4)

Bring to a final volume of 1L using deionized water. Adjust pH to 7.4 using either 1 N HCl or 1 N NaOH.

**Borate Buffer; pH 8.5**Boric Acid,  $H_3BO_3$ : 12.4 g/L (MW 61.8)

Sodium Tetraborate: 19.1 g/L (MW 381.4)

Add 50mL of (a) to 14.5mL of (b). Bring to final volume of 200mL using deionized water. Adjust final pH to 8.5 using 3 M NaOH solution.

**Carbonate-Bicarbonate Buffer; pH range 9.2 to 10.4**

0.1 M Sodium carbonate: 10.6 g/L (anhydrous; MW 106.0)

0.1 M Sodium bicarbonate: 8.4 g/L (MW 84.0)

mL Na Carbonate	4.0	9.5	16.0	22.0	27.5	33.0	38.5
mL of Na Bicarbonate	46.0	40.5	34.0	28.0	22.5	17.0	11.5
pH	9.2	9.4	9.6	9.8	10.0	10.2	10.4

Mix sodium carbonate and sodium bicarbonate solutions in the proportions indicated and adjust the final volume to 200mL with deionized water. Adjust the final pH using 1 N HCl or 1 N NaOH.

*Note: Low concentrations (0.05-0.1%) of antimicrobial agents, such as sodium azide or ProClin<sup>®</sup>, are often added to the storage buffer, particularly for long-term storage. Antimicrobials should be carefully selected, as they may exhibit differing stability, involve special disposal considerations, etc. For example, sodium azide may react with lead and copper plumbing to form explosive metal azides. Therefore, upon disposal of materials, large amounts of water must be used to flush the plumbing and prevent azide accumulation.*