WHITEPAPER

Buff Up on Buffers and Surfactants

BIOCARE MEDICAL

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Wash buffers are an integral part of immunohistochemical (IHC) procedures. They are used after each incubation step to wash away excess reagent from the specimen and maintain a constant pH by "soaking up" any free hydrogen (H+) ions. This feature helps maintain the morphological characteristics of antibodies and their epitopes, which enables specific binding. The saline and detergent content in buffer solutions also play a role in minimizing background staining.[2]

The two most common buffers in IHC are Tris-Buffered Saline (TBS) and Phosphate-Buffered Saline (PBS). TBS is often used in combination with Tween 20, which reduces nonspecific staining, helps reagents spread evenly across tissue, and may include a preservative (such as sodium azide) to prevent microorganism growth and increase shelf life. TBS is recommended for immunostaining of phosphorylated proteins, as PBS can interfere with "the interaction between phosphorylated proteins and its cognate phosphospecific antibodies."[1] However, TBS should be avoided in any method sensitive to the presence of amines.[1] Tris-based buffer is sensitive to changes in temperature. As the solution decreases in temperature, pH increases at a rate of approximately 0.03 units per degree centigrade.[2]

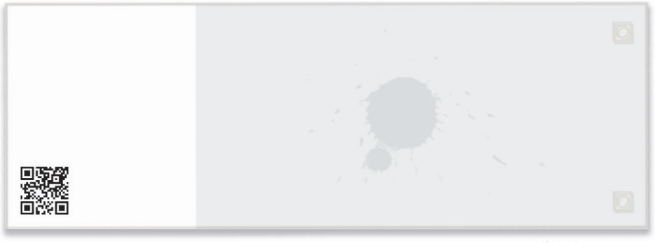
PBS is used frequently in cell culture.[1] Unlike TBS, PBS buffer's pH does not change significantly by temperature. However, PBS can reduce auto-fluorescence in immunofluorescent assays, and in some cases, this buffer can cause higher levels of nonspecific staining. It has also been observed to reduce the specific binding abilities of certain monoclonal antibodies (Anti-CD30, for example).[2] Additionally, PBS should not be used with alkaline phosphatase (AP) secondary antibody detections in IHC.

While many research laboratories prepare buffers from scratch, ready-to-use and concentrated buffers are readily available for immunohistochemical, immunofluorescent, and biochemical applications. Ready-to-use buffers are easy to use and save time for busy laboratories. Concentrated buffers need to be diluted to the proper working concentration before use but typically have a longer shelf life and save storage space. It is important to note pH is not determined by the concentration of buffer but by the ratio of the acid and base salt in the buffer. This ratio does not change when diluted. The higher the buffer concentration, the more acid or base that can be absorbed before a significant change in pH is observed. Once the buffer is exhausted, the pH is no longer controlled.

Biocare Medical offers a wide range of buffers and surfactants for both manual and automated staining. Most buffers are available in a concentrated format, and all can be stored at room temperature, saving precious refrigerator space. To determine which buffer is right for you, the experiment's requirements and limitations will need to be considered, along with the sample type, antibody source, and detection system.

Buffer Tween Slide Comparison

Buffer without Tween20 added. Notice the droplets pooling, which can lead to uneven distribution and uneven staining.



Buffer with Tween20 added. The full slide has even distribution, resulting in even, consistent staining.

Biocare Medical's Wash Buffers/Surfactants	Catalog Number	Product Description
PBS plus, 10X	PBS940M	• High quality PBS buffer with superior pH stability • Sodium azide and surfactant free; pH of 7.3 • For manual and automated histopathology and IHC applications with the addition of a surfactant
TBS plus, 10X	TBS942M	• Provides superior pH stability • Sodium azide, thimerosal, and surfactant free; pH of 7.6 • For manual or automated IHC applications with the addition of surfactant
Immunocare PBS, 10X	PWB941M	• High-quality PBS buffer with superior pH stability • Manual and automated IHC applications • Contains surfactant and allows for reagents to spread more uniformly across tissue section on slides.
Immunocare TBS, 10X	TWB943M	• Specially formulated for Alkaline Phosphatase (AP) detection systems; added enzyme activator to increase staining efficiency for AP systems. • Manual or automated IHC applications • Sodium azide and thimerosal free; contains a surfactant
TBS Automation Wash Buffer, 20X	TWB945M	• For manual, capillary gap, and automated IHC applications • Contains Tween 20 surfactant • Sodium azide and thimerosal free; pH of 7.7
Automation Tween 20, 20X	TWA20M	• Nonionic polysorbate detergent (surfactant) • Decreases background staining • May be diluted in deionized water for rinsing specimens on an automated staining system (such as intelliPATH FLX).
Tween 20	TWN20	• Detergent commonly added to buffers and reagents • For manual or automated staining systems.
SSC (Saline Sodium Citrate) tWash Buffer	BRI4039	• Ready-to-use buffer may be used as a post-hybridization wash buffer for in situ hybridization procedures.; pH 7.0-7.5 • Contains 0.3% NP40 and Proclin 950 as a preservative. • Contains 30 mM citrate (commonly identified as 2X SSC)

Feel free to call our knowledgeable Technical Support team to assist in making sure your protocol is perfectly optimized to meet the laboratory's needs. Contact Biocare anytime at 800-799-9499 or click the link here: https://biocare.net/products/ancillaries/wash-buffer-mounting-media/.

1. https://blog.atlasantibodies.com/how-to-succeed-with-your-ihc-buffers-chemicals

2. Immunohistochemical Staining Methods, Sixth ed. Dako Denmark A/S, An Agilent Technologies Company, 2013.