

## ADAPT-3D™ Immunostaining Buffer

Product Information  
Product No.: B673  
Storage: Ambient “room” temperature

### Product Description

#### Background:

The ADAPT-3D™ Immunostaining Buffer is a critical component of the ADAPT-3D™ Tissue Clearing Kit, designed to support effective and reliable immunolabeling of cleared tissue samples for high-resolution 3D fluorescence imaging. This buffer is formulated with bovine serum albumin (BSA) and normal donkey serum, both of which serve as blocking agents to reduce nonspecific antibody binding and background fluorescence during staining.

The combination of BSA and donkey serum provides a broadly effective blocking solution for many standard immunostaining protocols. However, due to species-specific interactions between antibodies and tissue antigens, some applications may require the addition of normal serum from the host species of the secondary antibody (e.g., goat, rabbit, or mouse). Incorporating species-specific serum can enhance blocking efficiency and improve signal clarity by further minimizing nonspecific binding.

*For optimal performance, it is recommended to tailor the blocking strategy based on the specific antibody panel and tissue type used in each experiment.*

#### Storage & Stability:

Upon arrival, store immediately at 2–8 °C. **Do not freeze**

#### Shipping:

The ADAPT-3D™ Immunostaining Buffer should be shipped with cold packs (2–8 °C) to maintain reagent stability during transit. Avoid freezing. For international shipments or extended transit times, insulated packaging with sufficient cold retention is recommended to ensure temperature integrity. *Note: Leinco has shown this product is stable for 72-96 hours at room temperature.*

#### Country of Origin:

USA

#### Directions for Use:

For researchers planning to perform immunolabeling, the 1X ADAPT-3D Staining Buffer (catalog# B673) is integral to the protocol. This buffer is specifically formulated to optimize

antibody penetration and minimize non-specific binding within cleared tissues, ensuring robust and precise fluorescent signals.

## **Procedure for Immunolabeling:**

### ***Initial Equilibration:***

Submerge your delipidated and rehydrated tissue samples in 1X ADAPT-3D Staining Buffer (catalog# B673). For smaller tissue sections (e.g., <5 mm), an incubation period of at least one hour at room temperature with gentle agitation is recommended to ensure adequate buffer penetration. For larger or whole tissues (e.g., intact organs or larger tissue blocks), an overnight incubation at 4°C with gentle agitation is advisable to facilitate thorough permeabilization and equilibration. This step is critical for preparing the tissue to optimally receive antibody penetration.

### ***Antibody Incubation:***

Following the initial equilibration, prepare your primary and/or secondary antibody solutions by diluting them to their optimized concentrations directly in fresh 1X ADAPT-3D Staining Buffer. Carefully transfer your tissue samples from the initial equilibration buffer into the prepared antibody solution. Incubate samples with the antibody-containing buffer for a duration appropriate for your specific antibodies and tissue type (typically overnight to several days at 4°C with gentle agitation, or as recommended by the antibody manufacturer for 3D tissue staining). Ensure complete immersion of the tissue.

### ***Washing Steps:***

After the antibody incubation, remove the antibody solution and thoroughly wash the samples with fresh 1X Wash buffer diluted from 5X concentrate (catalog# B674). Perform multiple washes (e.g., 3-5 washes, 1-2 hours per wash) at room temperature with gentle agitation to remove unbound antibodies and reduce background fluorescence. The number and duration of washes may need to be optimized based on the antibody and tissue characteristics. Utilizing the 1X Wash buffer diluted from 5X concentrate throughout these steps ensures the integrity of your cleared tissue, maximizes antibody specificity, and ultimately contributes to superior image quality in your 3D fluorescence microscopy.

*Each investigator should determine their own optimal working dilution for specific applications. See directions on lot specific datasheets, as information may periodically change.*

## **Hazard Description:**

The 1X ADAPT-3D™ Immunostaining Buffer contains bovine serum albumin (BSA) and a low concentration of sodium azide, a preservative that may pose health and environmental risks if not handled properly. Sodium azide is toxic if ingested, inhaled, or absorbed through the skin and may react with heavy metals to form explosive compounds. Proper laboratory safety practices must be followed when handling this reagent, including the use of gloves, lab coat,

and protective eyewear. Dispose of solutions containing azide in accordance with local, state, and federal regulations.

For detailed safety, handling, storage, and disposal information, refer to the Safety Data Sheet (SDS) provided with the product.

**Related Products:**

Product No.	Description
B676	ADAPT-3D™ Delipidation Buffer Component B
B672	ADAPT-3D™ Delipidation Buffer Component A
B671	ADAPT-3D™ Decolorization Buffer
B674	ADAPT-3D™ 5X Wash Buffer
B675	ADAPT-3D™ Refractive Index Match Buffer
A630	ADAPT-3D™ Tissue Clearing Kit