

# ONI STORM Imaging Buffer Preparation Protocol

## Overview

This protocol describes preparation and handling of the ONI STORM imaging buffer, including proper resuspension of Component B and final mixing with Component A. The STORM buffer is critical for optimal fluorophore blinking behavior during super-resolution imaging.

## Materials

- Component A (buffer stock)
- Component B (lyophilized reagent in white tube)
- Resuspension buffer (provided with Component B)
- Pipettes and low-retention tips
- Ice bucket

## 1. Resuspension of Component B (if not already prepared)

If Component B has not yet been resuspended, it will be in a white tube containing lyophilized reagent along with a separate resuspension buffer.

- Add 25  $\mu\text{L}$  of the provided resuspension buffer to the lyophilized Component B.
- Place the tube on ice and allow it to sit for 5 minutes to fully dissolve.
- Gently mix by pipetting up and down. Avoid introducing air bubbles, as this can affect buffer performance.

## 2. Preparation of Working STORM Buffer

- Thaw Component A completely before use.
- Mix Component A and Component B at a ratio of 100  $\mu\text{L}$  A to 1  $\mu\text{L}$  B.
- Prepare only the volume needed for immediate use.

## 3. Volume Guidelines

- For EV chip experiments: use approximately 20  $\mu\text{L}$  of prepared STORM buffer.
- For glass-bottom dishes: add sufficient buffer to fully cover the sample surface.

## **4. Storage After Preparation**

After preparing the working buffer, return both Component A and Component B stock solutions to the freezer immediately to preserve activity.

## **Notes and Best Practices**

- Always keep Component B and resuspended solutions cold during handling.
- Avoid introducing bubbles when mixing, as oxygen can reduce STORM imaging efficiency.
- Do not prepare excess buffer; freshly mixed buffer yields the best imaging results.