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.

a. Add 25 µL of the provided resuspension buffer to the lyophilized Component B.

b. Place the tube on ice and allow it to sit for 5 minutes to fully dissolve.

c. Gently mix by pipetting up and down. Avoid introducing air bubbles, as this can affect buffer performance.

2. Preparation of Working STORM Buffer

a. Thaw Component A completely before use.

b. Mix Component A and Component B at a ratio of 100 µL A to 1 µL B.

c. Prepare only the volume needed for immediate use.

3. Volume Guidelines

- For EV chip experiments: use approximately 20 µL 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.