



## Protocol for Preparation of dSTORM Imaging Buffer

This protocol can be used to prepare imaging buffer for super-resolution dSTORM imaging applications.

### Stocks

- Buffer A: 50 mM Tris-HCl (pH 8.0) + 10 mM NaCl- store at room temperature
- Buffer B: 50 mM Tris-HCl (pH 8.0) + 10 mM NaCl + 10% (w/v) glucose- store at 4 °C
- 1 M MEA: 77 mg MEA dissolved in 1 mL Buffer A- store at 4 °C
- Gloxy: glucose oxidase (Gluox) + catalase mixture dissolved in buffer A- store at 4 °C

When making Gloxy stock, calculate the amount to add based on the active units (AU) since not all the protein in the bottle is active. Both the Catalase and Glucose from Sigma should have the information written on the tube. For 50x stock of Gloxy mix 8440 AU Gluox + 70200 AU Catalase into 1mL of 50 mM Tris + 10 mM NaCl (pH 8.0) and vortex.

### Materials

- Cysteamine (MEA) #30070-10G (Sigma)- store at 4 °C
- 2-Mercaptoethanol #63689-25ML-F (Sigma)- store at 4 °C
- Glucose oxidase type seven from Aspergillus #G2133-50KU (Sigma)- store at -20 °C
- Catalase from Bovine liver C40-100 mg (Sigma)- store at -20 °C
- 1M Tris pH 8.0 # 22638-500ML (Affymetrix / USB)- store at room temperature
- Sodium Chloride (NaCl) #S7653-250G (Sigma)- store at room temperature
- D-Glucose # G8270-1KG (Sigma)- store at room temperature

### Procedure

#### Imaging buffer

1. Mix 50  $\mu$ L of 1 M MEA + 20  $\mu$ L of 50x Gloxy + 930  $\mu$ L of buffer B in a 1.5 ml centrifuge tube and vortex.

Make the mixture on ice or at 4 °C fresh before imaging. Typical Concentration: 50 mM MEA 1x Gloxy in buffer B.

#### Imaging buffer for multicolor imaging

2. Mix 20  $\mu$ L of 1 M MEA + 10  $\mu$ L of 2-Mercaptoethanol + 20  $\mu$ L of 50x Gloxy + 950  $\mu$ L of buffer B in a 1.5 ml centrifuge tube and vortex

Make the mixture on ice or at 4 °C fresh before imaging Typical Concentration: 20 mM MEA + 1% (v/v) 2-Mercaptoethanol + 1x Gloxy in buffer B Buffers can be used at room temperature imaging for approximately 2 hours.

### Bruker Fluorescence Microscopy

Madison, WI • USA  
Phone +1.608.662.0022  
productinfo@bruker.com