**STORM Imaging Buffers**

**2-mercaptotheanol:** Sigma Aldrich #63689-25ML-F

**Cysteamine (MEA):** Sigma Aldrich #30070-10G

**Glucose Oxidase from Aspergillus niger-Type VII, lyophilized powder, >100,000 units/g solid:**

 Sigma Aldrich #G2133-10KU

**Catalase from bovine liver-lyophilized powder, >10,000 units/mg protein:**

Sigma Aldrich #C40-100MG or CalBiochem #21901-5MU

**1M Tris pH 8.0:** Life Technologies #AM9855G

**1N HCl:** Electron Microscopy Sciences #16770

**Phosphate-Buffered Saline (PBS), 1x:** Electron Microscopy Sciences #19344-10

**NaCl:** Sigma #S9888-25G

**Solutions:**

**\*1M MEA (4ml):** recommended 77mg MEA + 1.0ml 0.25N HCl. Increased by 4x amount

 **= 0.308g MEA + 4ml 0.25N HCl**

Good for 1 month at 4oC

4.0 ml of 0.25N HCl = 1ml 1N HCl + 3ml H2O

**\*GLOX Solution (1.75ml) :** recom. 14mg Glucose Oxidase + 50ul Catalase (17mg/ml) + 200ul Buffer A

1 vial has 7x the amount needed, so multiply amounts by 7-fold.

 **1 vial Glucose Oxidase 10KU + 350ul Bovine Catalase (17mg/ml) + 1.4ml Buffer A**

Spin down at 14K rpm, use only supernatant. Store 4oC for 2 weeks.

 **Glucose Oxidase:** 10KU per vial = 10,000Units / 100,000units/g = 0.10g or 100mg/vial

Need 14mg, so 1 vial has about 7x the amount needed.

**Bovine Catalase:** 5MU per vial = 5,000,000Units / 10,000units/mg = 500mg/vial

Need 17mg/ml, so vial has about 30x the amount needed.

Dissolve 1 vial 5MU Bovine Catalase in 30ml H2O = 17mg/ml

**\*Buffer A (10ml): 10mM Tris (pH 8.0) + 50mM NaCl**

 **100ul 1.0M Tris (pH 8.0) + 500ul 1.0M NaCl + 9.4ml H2O**

1M NaCl (10ml) = 0.58g / (58.44g/mole)(0.01L), so dissolve 0.58g NaCl in 10ml H2O = 1M NaCl

 (0.010M Tris)(0.010L)/(1.0M Tris) = 0.0001L = 100ul 1.0M Tris pH8 in 10ml

 (0.050M NaCl)(0.010L)/(1.0M NaCl) = 0.0005L = 500ul 1.0M NaCl in 10ml

**\*Buffer B (10ml): 50mM Tris (pH 8.0) + 10mM NaCl + 10% Glucose**

 **500ul 1.0M Tris (pH 8.0) + 100ul 1.0M NaCl + 1 g Glucose + 9.4ml H2O**

(0.050M Tris)(0.010L)/(1.0M Tris) = 0.0005L = 500ul 1.0M Tris pH8 in 10ml

 (0.010M NaCl)(0.010L)/(1.0M NaCl) = 0.0001L = 100ul 1.0M NaCl in 10ml

Weight (g) \* 100/Volume (ml) = %, so (10% Glucose \* 10ml) / 100 = 1 gram Glucose

**Method A: recommended for ATTO 488 and Alexa 568. If using either with Alexa 647, use Method A.**

STORM Imaging Buffer with MEA

1. On ice, add 7ul GLOX + 70ul MEA + 620ul Buffer B.
2. Add 700ul imaging buffer per well of 8-wll Lab-Tek
3. Samples can be used in imaging buffer for several hours.

**Method B: recommended for Alexa 647 and Cy3B**

STORM Imaging Buffer with 2-mercaptoethanol

1. On ice, combine 7ul GLOX + 7ul 2-mercaptoethanol + 690ul Buffer B.
2. Add 700ul imaging buffer per well of 8-wll Lab-Tek
3. Samples can be used in imaging buffer for several hours.