Preparation of Glucose Oxidase/Catalase

Protocol modified from *Nikon Super Resolution Microscope N-STORM Sample Preparation Manual* (PDF found at http://www.mvi-inc.com/wp-content/uploads/N-STORM+Protocol.pdf)

Purpose: use 2uL/mL to remove free radicals produced by exciting fluors, which could shear DNA if left in solution

Materials:

- **T50 Buffer** (Cold Spring Harbor) – 10mM Tris-Cl (pH=8.0), 50mM NaCl
  - In practice, using Tris-Cl, pH=7.5 still works
- **Catalase** – solid (stored @ -20°C)
- **Glucose Oxidase** – lyophilized powder (stored @ -20°C)

Overview:

1) create stocks of glucose oxidase and catalase
2) combine to create GLOX solution
3) incubate at 4°C
4) centrifuge and remove precipitate
5) filter and store

Protocol:

- Suspend catalase at 17 mg/mL in T50 buffer with 50% glycerol at 4°C
  - 17mg + 625uL 80% glycerol + 325uL T50 buffer
  - If using 1x T50 buffer, this will be diluted, but that does not seem to affect GLOX
- Suspend 28mg of Glucose Oxidase in 400uL of T50 buffer at 4°C (vortex)
- Add 100uL of Catalase solution to re-suspended glucose oxidase (500uL total)
- Incubate at 4°C for 20 minutes (catalase activates glucose oxidase)

- Spin down precipitate at 14,000 rpm in a table-top centrifuge for 5 minutes at 4°C
  - If no pellet is visible after centrifuging: flash-freeze the 500uL GLOX solution, let thaw, and then centrifuge again to pellet precipitate
- Filter the supernatant with a 0.22um syringe filter
- Store @ 4°C if using immediately (manual says GLOX is good at 4°C for two weeks) flash freeze and store long-term stocks of GLOX at -80°C