Here's the blinking buffer protocol. Remember:

Aliquots are good for only 2-3 weeks and the buffer is mixed just before imaging.

Blinking buffer oxidizes and we need to seal them for best results during imaging.

Below is the protocol for blinking buffer

Fixation and permeabilization for primary ab staining like normal.

Concentration for staining should start at 1mM and then go up from there to find the right concentration.

We should expect to try a multiple concentrations to find the optimal for your samples.

**This is what you need to make GLOX buffer:**

Ingredients need to be fresh

**TUBE 1 Glucose Buffer**

5g Glucose

50ml 100 mM Tris-HCl buffer (pH 8.0)

**TUBE 2 Catalzase**

80 mg glucose oxidase

-12.8 mg catalase

**TUBE 3 MEA**

You will want a few aliquots of different volume for quickly adjusting concentrations to start.

Weigh out individual portions (~20-100 mg) of cysteamine in 1 ml reaction tubes and label them with exact weight. I would weigh out 20mg, 25mg, 30mg, 35mg, ...out to 100mg

We will dilute from there.

**Here is the protocol:**

In MINFLUX imaging cyanine dyes are a common labelling choice. To enable switching of the dyes, a buffer containing a reducing agent - such as cysteamine (MEA) - and an oxygen scavenging system is applied. This is often an enzymatic system containing glucose oxidase and catalase (commonly referred to as GLOX, or a GLOX buffer).

Preparation of GLOX buffer:

**TUBE1: BLINKING BUFFER BASE**

(Store at 4°C)

1. To 50 ml of 100 mM Tris-HCl buffer (pH 8.0), add 5 g glucose.
2. Mix until dissolved and store in fridge up to 2 weeks.
3. Optional: sterile filter for longer shelf life

**TUBE 2: 20x CATALASE ENZYME MIX**

(store at -20° for long term or at 4° for maximum 1 month)

1. Weigh in a 15 ml falcon tube:

- 80 mg glucose oxidase

-12.8 mg catalase

1. Add 5 ml 100mM Tris-HCL buffer pH 8.0 (without glucose), and 5 ml glycerol to the tube. Mix until dissolved into a clear yellow liquid.
2. Divide into 500 µl aliquots in small reaction tubes and store in -20°C freezer up to 2 weeks

**TUBE 3: MEA**

(Store at 4°)

1. Let cysteamine (MEA) warm up to room temperature to prevent it collecting moisture.
2. Weigh out individual portions (~20-100 mg) of cysteamine in 1 ml reaction tubes and label them with exact weight. Seal tubes with Parafilm and store in the fridge up to 2 weeks.
3. Dilute with PBS to 1M stock just before use (77 mg/ml)
4. Store diluted stocks at 4°C for up to 1 week.

Right before imaging, prepare the blinking buffer out of TUBES 1, 2 and 3:

95 µl blinking buffer base (TUBE 1)

5 µl Enzyme mix (TUBE 2)

Depending on the sample, 1-10 µl MEA (TUBE 3)

Composition of the final buffer (GLOX):

100 mM Tris-HCl pH 8.0,

10% Glucose

0.4 mg/ml Glucose Oxidase = 40 U/ml

64 µg/ml Catalase = 130-320 U/ml

10-25 mM MEA

Blinking buffers should be prepared fresh before imaging in an environment sealed against oxygen. The buffer can function for between 4 and 8 hours in an air tight environment.

--> PLEASE NOTE that the concentration of the reducing agent should be optimized for your particular fluorophore and the fluorophore density in your sample for best results. This is why we start with so many aliquots.