

Immobilization of Samples on Slide

1. For a PEGylated slide, use 1X PBS sterile filtered buffer solution.
2. Wash the mPEG/PEG-biotin using 1X PBS. Flow on 20 μ L of the PBS using a 20 μ L tip. The solution is viscous and takes some time to wash it out. Wick up the excess using filter paper.
3. Wash the channel with an additional 200 μ L of 1X PBS. This can be done with a 200 μ L tip by adding the solution on top of the slide hole and sucking up the solution at the other end of the channel with filter paper. Watch out for air bubbles. If you get an air bubble and can't flow any more buffer through, use a smaller tip to add buffer directly into the slide hole until the air bubble is pushed through the channel.
4. Wash all the channels on all the slides planned for use.
5. Make a 1:40 - 1:50 dilution of Streptavidin from the 10 μ L aliquot in the -80°C freezer. (10 μ L into 400 μ L PBS final)
6. Flow on 60 μ L of the Streptavidin solution and let it incubate on the slide for 2 minutes.
7. Wash away the Streptavidin with 1X PBS. Flow on 20 μ L first to get rid of most of it quickly, and then thoroughly wash with 200 μ L of 1X PBS.
8. Prepare sample to be in the range of 10-100 pM of the fluorescent molecule.
9. Prepare a wash buffer solution with oxygen scavenging solution (OSS). Dilute each component of the OSS (aliquots of Glucose Oxidase and Catalase are 100x stocks stored in the -80°C, 2.5M (45%) glucose solution stored at RT) in 1X PBS. I usually make a 200 μ L stock and add 2.0 – 3.0 μ L of each. It is important to add the OSS to wash the sample, because OSS helps to reduce photobleaching by singlet oxygen. Sometimes it is also useful to add Trolox (also in -80°C, 1:100 dilution from 100mM stock -- 3.0 μ L into 100 μ L, ~3mM) to reduce blinking.
10. Flow on 60 μ L of the sample (or enough to fill the channel), again wick away excess with filter paper.
11. Let it sit for 2 minutes at RT.
12. Wash away with 200 μ L of 1X experimental buffer + OSS.
13. Repeat sample conjugation and washing with all lanes. Remember to do at least two negative controls to assess nonspecific binding.
14. Slides are ready to put on the microscope!

Hoskins Lab	Streptavidin	SR #: srAAH0XXX
Solution Recipe		Revision Date: 2017/03/28
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I. **RF1**

This recipe card will explain how to make Streptavidin, 10uL aliquots.

II. **Safety**

N/A

III. **Materials**

SA10-10mg – Prozyme Streptavidin (-20C freezer in dry box)

MQ H₂O

IV. **Recipe**

	1mL
Streptavidin (mg)	10
MQ H ₂ O (mL)	1

V. **Notes**

After dissolving, prepare 10μL aliquots and freeze at -20°C.

Hoskins Lab	100X Catalase	SR #: mrAAH0008
Solution Recipe		Revision Date: 2013/11/25
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I. 100X Catalase

This recipe card will explain how to make 100X Catalase

II. Safety

N/A

III. Materials

C-40 Sigma Catalase (-20C freezer in dry box)

Syringe filter

300 kDa Millipore Filter

H₂O

IV. Recipe

	1 mL	2 mL	5 ml	10 mL
Catalase (U)	150,000	300,000	750,000	1,500,000
H ₂ O (mL)	1	2	5	10

- Syringe filter at 0.22 μm
- Spin at 7K for ~ 30 min through 300 kDa Millipore filter at 4C
- Prepare in 10 μL aliquots
- Quick Freeze in liquid nitrogen
- Store at -80°C

V. Notes

The above Millipore filter is cat. No: UFC4 TMK 25, polysulfone membrane with 300,000 nominal molecular weight cutoff...but I cannot find them on the Millipore site. You can use any equivalent filter. I think that 300 kDa filter step has been skipped sometimes in preparing the catalase, but the solution is visibly dirtier.

Hoskins Lab	100X Glucose	SR #: srAAH0016
Solution Recipe		Revision Date: 2013/11/25
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VI. **RF1**

This recipe card will explain how to make 100X glucose which can be used in a OSS solution.

VII. **Safety**

N/A

VIII. **Materials**

D-Glucose, anhydrous Fisher Scientific 50712744 (dry chemicals, sugars)

MQ H₂O

IX. **Recipe**

	1mL	5mL	7mL	10mL
Glucose (g)	0.45	2.25	3.2	4.5
MQ H ₂ O (mL)	1	5	7	10

X. **Notes**

Solution should be heated in the microwave to dissolve. After dissolving, prepare 200µL aliquots and freeze at -20°C.

Hoskins Lab	100X Glucose Oxidase	SR#: srAAH0017
Solution Recipe		Revision Date: 2017/03/28
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I. **RF1**

This recipe card will explain how to make Cathode buffer for Tricine SDS-Page.

II. **Safety**

III. **Materials**

20mM Tris Acetate, pH 8

Glucose Oxidase (Sigma G2133-10KU) (-20C freezer in dry box)

Glycerol

IV. **Recipe**

	2.4mL
20mM Tris Acetate (pH 8, mL)	1.2
Glucose Oxidase (U)	10,000
Glycerol 100 (mL)	1.2

V. **Notes**

Glucose oxidase comes in bottles of 10,000 units, so the tris acetate and glycerol can be added directly to the glucose oxidase bottle. The solution should be kept on ice throughout. Glucose Oxidase should be filtered with a 0.22µm syringe filter. It should then be prepared in 10µL aliquots and quick frozen in liquid nitrogen. Store samples at -80°C.

Hoskins Lab	Trolox	SR #: srAAH0XXX
Solution Recipe		Revision Date: 2017/03/28
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VI. **RF1**

This recipe card will explain how to make Trolox, 20uL 100mM aliquots.

VII. **Safety**

N/A

VIII. **Materials**

238813-1G Sigma Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (4C deli case)

DMSO, Fisher Scientific, NC0436450

IX. **Recipe**

	1mL	2mL
Trolox (mg)	25	50
DMSO (mL)	1	2

X. **Notes**

After dissolving, prepare 20μL aliquots and freeze at -20°C.

Hoskins Lab	100X OSS Solution	SR #: srAAH0021
Solution Recipe		Revision Date: 2013/11/25
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I. **100X OSS Solution**

This recipe card will explain how to make 100X OSS Solution

II. **Safety**

N/A

III. **Materials**

100X Glucose

100X Catalase

100X Glucose Oxidase

IV. **Recipe**

Add the glucose, catalase, and glucose oxidase (from their separate aliquots) to the experimental buffer.

V. **Notes**

It works to suspend both the catalase and glucose oxidase into just H₂O or your experimental buffer and/or to use 50% glycerol for both. For example, the recipe from the Vale lab uses 2X their experimental buffer and then adds 50% glycerol so that the addition of the scavenger does not contribute to altering their salt concentrations. These enzymes seem to be robust and all these combinations appear to work.

I typically take a new aliquot of both the glucose oxidase and catalase every day, but refreezing the aliquots also works ok: I will often attach my dsDNA template to the slide using buffer containing glucose oxidase and catalase from my last experiment (i.e. refrozen once) and thaw the new aliquots only when it is time to actually run my experiment.