

## **On-resin IgG labeling with Alexa fluor NHS esters**

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Protocol for labeling 5-100 $\mu$ g IgG

### *Benefits:*

Works with small amounts of IgG, exact amount doesn't matter

Works if the IgG is diluted in BSA, detergent etc.

So, good for labeling commercial antibody where you don't know the exact amount of what else has been added.

High dye/protein ratio

Low contamination with free dye

Seems to work well with all antibodies tested so far including a mouse monoclonal anti-tubulin antibody.

### *Notes:*

The amounts of dye added (1mM 2x20 $\mu$ l) aims for high Alexa dye/IgG ratio, which is suited for indirect immunofluorescence. You can adjust the dye concentration downwards if you prefer less dye/protein, or are using sticky dyes like TMR etc.

The Dye-NHS ester is added to a final concentration of 1mM independent of how much antibody is present. Most of this dye gets hydrolyzed.

This method should work for any NHS ester, eg biotin.

### *Resin:*

Protein G (Pierce) works for rabbit and mouse IgG. Protein-A Affiprep (Biorad) works for rabbit and goat IgG but not mouse. Yields, labeling were similar for rabbit IgG using these two resins

40 $\mu$ l slurry is ~25 $\mu$ l packed resin. In principle this can bind up to ~250 $\mu$ g of IgG.

### *Fluor:*

Dissolve Alexafluor NHS ester (Mol Probes) in dry DMSO at 50mg/ml. Store in aliquots at -20 $^{\circ}$  in a tube with some drierite or silica gel to keep dry. Its ok to freeze-thaw an aliquot a few times, but try to keep it dry.

50mg/ml is ~0.7M for A488, ~0.5M for A568 and A647, but this may be batch dependent. If you want to be accurate you cannot trust weight for these dyes, you have to use optical adsorbtion to measure concentration. I use epsilon values (ie the OD of a 1M solution at the max absorbtion) of 80,000 for 488, 120,000 for 568 and something like 200,000 for A647.

### *Protocol:*

All steps at RT except the final collection of eluate on ice.

Packing, loading and washing done by gentle pressure with a 200microl pipette.

Use 100microl per wash. Each wash should go through in ~60sec

*Making the tip:*

Take a 200 $\mu$ l pipette tip

Flame the end with a Bunsen burner just enough to seal the tip closed – not a big blob of plastic. This takes less than 1sec in the flame – just wave the tip through a couple of times.

Cut through the sealed end with a *thin* razor blade – the shaving kind, not the lab kind. The resulting slit will stay closed and hold the resin in place.

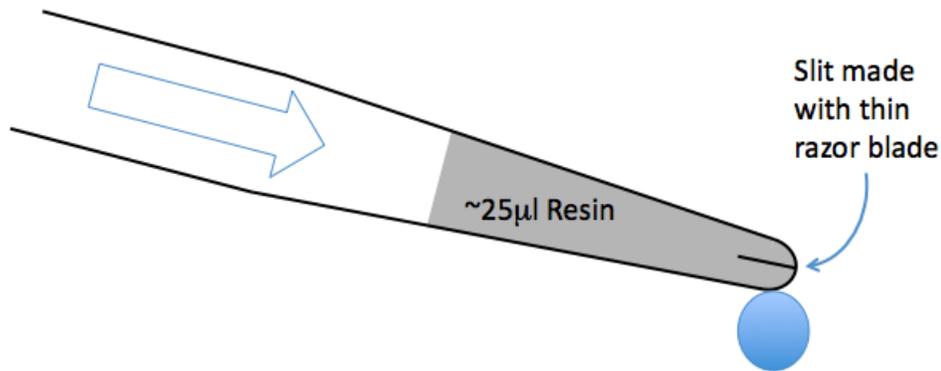
Add 40 $\mu$ l of Protein-G Ultralink resin slurry (Pierce) for ~25 $\mu$ l of packed resin.

Use a cut off tip and swirl the resin slurry well before you pick it up.

Wash x2 HBS (0.15M NaCl, 20mM KHEPES 7.7)

Make sure the resin is staying in the tip! If the slit is too open it will come out.

This is usually because the razor blade used to make the slit was too thick.



Add antibody to top of resin. Should be in a buffer like HBS. Can dilute with HBS to ~100 $\mu$ l final before adding.

Push antibody through resin 5 times using gentle pressure – ie collect the flow through and re-load it 5x. Should not go through too fast.

*Note: Test with labeled IgG suggest ~1/2 the antibody binds on each pass through the resin bed.*

Wash x2 HBS

Wash x2 0.2M KHEPES 7.7. Leave top of resin dry.

Take 20 $\mu$ l 0.2M KHEPES 7.7

Add: 0.3 $\mu$ l A488-NHS, 0.4 $\mu$ l A568-NHS and 0.4 $\mu$ l A647NHS to give a labeling reaction that is ~1mM Dye-NHS

Add dye, mix, and load **immediately** onto resin. The dye starts to hydrolyze!

Push dye mix through the resin bed, leaving just a little on top. This should completely saturate the resin, and some dye should come out of the tip. If you used more resin, you would need a larger volume of 1mM dye.

Incubate RT 10-30min

*Note – the dye undergoes hydrolysis with a half-life of ~5min at pH 7.7, so the reaction is finished in ~15min.*

Repeat the labeling once more using freshly diluted Alexa Dye NHS  
Incubate RT 10-30min

Wash x2 0.2M KHEPES 7.7

Wash x6 HBS

There should be no color in the flow through after the first two washes. All the resin will be labeled. Note that most of the dye is attached to the protein G, not the bound IgG, and will not elute.

Put 5 $\mu$ l aliquots of 1M TrisCl pH 9.0 into ~6 tubes on ice. This is to rapidly neutralize the acidic eluate. 1 volume of Tris pH 9.0 will neutralize 4 volumes of 0.2M acetic acid.

Elute with sequential 20 $\mu$ l aliquots of 0.2M acetic acid at RT. I start with 10 $\mu$ l in the first elution and try to have no eluted protein in the first tube. Most of the eluate is in tubes 2 and maybe 3. Collect the eluted droplets into the tubes with TrisCl in them and mix as soon as possible.

Pool the tubes with a lot of color in them, or keep separate if you want a high concentration tube. I generally pool tubes 2 and 3 only.

Store labeled IgG at 4 $^{\circ}$ .

The Tris-acetate buffer present after elution seems to work fine for antibody storage for months, and may retard bacterial growth. You can add azide to 0.1% for long term storage.