Labelling Monoclonal Antibodies with Long-Armed Biotin

Please note – this protocol has been optimised for certain conditions and is meant as a guide only, you may need to alter some parameters to suit your own experiment

1. Use purified monoclonal antibody at 1 - 4 mg/ml; antibodies more dilute than 1 mg/ml will show sub-optimal labelling
2. Dialyse against bicarb buffer at 4°C. Dialyse in a volume of at least 1 litre for 5 ml of antibody, with 1 change of buffer over 1 - 2 days. This will raise the pH to 8.4 and remove free $NH4^+$ ions and azide. Determine antibody concentration after dialysis (concentration antibody in mg/ml = A_{280} x 0.74 x dilution factor).
3. Biotinylation reagent (amino-hexanoyl-biotin-N-hydroxysuccinimide; Zymed) **must be allowed to reach room temperature before opening**. Note: the reagent should be stored *desiccated* at 4°C
4. Dissolve biotinylation reagent to 10mg/ml in anhydrous DMF (dimethylformamide). This must be done just prior to labelling, as the solution can't be stored. For most purposes only small amounts of the solution are required, so it is best to weigh an empty 5 ml glass bottle (DON'T use plastic), add a small amount of biotinylation reagent to the tube (one small spatula-full is fine), then weigh again and calculate the amount of reagent added. Then make up in the appropriate volume of DMF.
5. Add 10 µl of biotin solution for every 1 mg of antibody and mix on a rotator for 2 hours at room temperature.
6. Remove unbound biotin by one of the following methods:

- Size-exclusion chromatography on a Sephadex G-25 column (eg 'PD-10')

  - Equilibrate column with 5 ml of PBS/1% BSA (to block non-specific protein-binding sites), and then wash with 20 ml of PBS.

  - Allow the level of PBS to fall to the top of the gel bed, then add labelled antibody in a volume of 2 ml - 2.5 ml (if presently at less than 2 ml, first dilute in carbonate/bicarbonate buffer).

  - Begin collecting 0.5-1 ml fractions, then when the level of liquid falls to the top of the gel bed, elute with 5 ml of PBS. The labelled antibody should come off in about the 2nd and 3rd mls off the column.

  - Determine A_{280} of the fractions. Note that unbound biotin also absorbs at 280 nm, so will see 2 peaks of absorbance, usually quite close to each other. Pool fractions in the first peak.

  - The column can be stored for re-use by washing thoroughly in PBS/0.01% azide.

or...

- Dialyse against 1 litre of PBS at 4°C with 2 -3 changes over 2 days

7. Add sodium azide to 0.04% and store at 4°C, protected from light. Ideally, antibodies should be stored at at least 1mg/ml; if they are significantly more dilute than this, add BSA to 1% to help prevent the formation of aggregates and passive adsorption of the antibody to the walls of the tube.

Note: the formation of aggregates can increase background staining by allowing binding to Fc receptors. Aggregates can be removed by ultracentrifugation (eg 15 mins at 100 000 x *g*)

Note: the final antibody concentration, and the extent of labelling, cannot be determined by absorbance readings, as biotin also absorbs at 280 nm. Therefore assume that the concentration is the same as that determined prior to labelling, and test the success of labelling using a one-site ELISA (ie coat tray with biotinylated antibody, block, incubate with streptavidin-HRP, add substrate)

Bicarb Buffer:

	(to make 1 L)	*(to make 2 L)*
0.1 M $NaHCO_3$	8.4 g	16.8 g
0.1 M NaCl	5.84 g	11.68 g
in distilled water		
pH to 8.4		