Antibody labelling

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Antibody labeling procedure with EZ-Link Plus Activated Peroxidase kit (adapted from Pierce; pH9.4 protocol)

Last modified 06/30/08

- Buffer exchange (Zeba Desalt Spin columns)

    [For sample volumes of 500-2000ul use 5ml columns & 15ml collection tubes; for sample volumes of 700-4000ul use 10ml columns & 50ml collection tubes]

1. Prepare buffer by adding 500ml of ddH2O to the dry-blend BupH Carbonate-Bicarbonate buffer from the Ab labeling kit.
2. Twist off the column's bottom closure and loosen cap. Place column in a collection tube.
3. Centrifuge column at 1000 xg for 2mins to remove storage solution.
4. Add buffer to the column and centrifuge at 1000xg for 2mins to remove buffer. (For 5ml columns use 2.5ml of buffer; for 10ml columns use 5ml of buffer.) Repeat this step twice.
5. Place column in a new collection tube, remove cap and slowly apply sample to the center of the compact resin bed.
6. (Optional) for low volumn samples apply a stacker of ddH2O or buffer to the resin bed after the sample has fully absorbed.

    [40ul stacker for samples <350ul in 2ml columns; 100ul stacker for samples <750ul in 5ml columns; 200 stacker for samples <1500ul in 10ml columns.]
7. Centrifuge at 1000 xg for 2mins to collect the sample. Discard column after use.

- Labeling

8. Prepare 1ml of IgG in 0.5-1.0ml of Carbonate-Bicarbonate buffer.
9. Reconstitute 1mg of lyophilized activated peroxidase with 100ul ddH2O and add it to the IgG solution or add the protein sample directly to the lyophilized activated peroxidase.
10. Incubate reaction for 1 hour at RT.
11. In a fume hood, add 10ul of sodium cynaborohydride and react at RT for 15mins.
12. Add 20ul of quenching buffer and react at RT for 15mins.
13. (Optional) If background problem persists during sandwich ELISA applications with this Ab conjugate, proceed to Step 1 of purification section.
14. Store conjugate at 4C for up to 4 weeks. For long-term storage, add either 10mg/ml BSA or an equal volume of glycerol. Prepare single-use aliquots and store at -20.

- Purification (For purified IgG only; this part of the protocol was theoretically based on principals of Pierce conjugate purification kit 44920 but constructed de novo)

15. Add 2ml of Ni-NTA agarose beads to conjugate in a 50ml flask and agitate at 4C for a 1 hour.
16. Load the mixture onto a column and let it settle, collecting the flowthrough. Determine binding efficiency by comparing it with the original sample.
17. Wash column 3x 4ml wash buffer.
18. Elute 1x with 0.5ml of elution buffer and 6x 1ml elution buffer, collect the flow-through. These fractions should contain the conjugate.
19. If desired, concentrate/dialyze/desalt the conjugate or exchange it into another buffer.

Note:

20. Samples need to contain >20ug/ml protein to be used with Zeba desalting column. Retention rate

is 95%.

21. Varying conjugation parameters, such as molar excess of peroxidase, buffer, and pH affects HRP incorporation level. Polymeric conjugates with high enzymatic activity are formed at high pH, while lower molecular weight conjugates are formed at near-neutral pH.
22. Do NOT use NaN3 as a preservative for buffers or the conjugate.
23. High phosophate []s will strip some Ni2+ ions off the column causing some conjugate to leak from the column. For best results use other buffers e.g. TBS. If the sample is in PBS, applying columns of 1-2ml will prevent conjugate leakage.
24. Performing dialysis instead of desalting may reduce conjugate loss.

Instructions from Pierce:
link antibody with an enzyme

For antigen immobilization - 44894
mix antibody and run through column
For elution of antibodies use igG elution buffer-21004

dialyze it into PBS

or add 1M tris pH8 1:10 ratio immediately to neutralize the harsh elution buffer conditions (can run through a de-salting column)
for easier antibody labelling - 31489

note: tris react with HRP, antibody labelling has to be done in PBS/carbonate buffer.