Indirect labeling of protein with ^{125}I

Indirect radiolabeling with ^{125}I is a method for first conjugating ^{125}I to a linker, and then conjugating the ^{125}I-linker product to a protein of interest. This method makes it possible to label proteins on lysine residues, in contrast to the tyrosine residues being targeted in direct labeling procedures. Indirect labeling often results in lower specific activity than direct labeling. This protocol describes how proteins can be indirectly labeled with ^{125}I, suitable for runs in LigandTracer® Grey. The protocol may also be used for ^{123}I, ^{124}I and ^{131}I, detectable with LigandTracer Yellow (all three) and LigandTracer White (^{124}I and ^{131}I).

Important information

Possession and handling of radioactive material may require licenses and/or special training according to national or local regulations or laws. Do not follow this protocol unless all legal requirements regarding possession and handling of radioactive material are met.

Note that ^{125}I will be conjugated to the lysine residues and may affect the binding properties of some proteins.

Materials

- Protein (preferably at least 0.5 mg/ml in stock solution): 10-300 µg
- ^{125}I
- Chloramine-T (CAT)
- Na_2SO_5
- Gel filtration column, e.g. NAP™-5
- PBS
- N-succinimidyl-para-(trimethylstannyl)-benzoate (SPMB)
- 5 % Acetic acid in methanol
- 0.1 % Acetic acid
- Borate buffer, pH 9

Procedure

1. Prepare the CAT and the Na_2SO_5 solutions by dissolving them in MilliQ water to obtain a concentration of 4 mg/ml of each. The solutions should be used within an hour and then discarded.
2. Dissolve SPMB in 5 % Acetic acid in methanol to a concentration of 1 mg/ml. The SPMB solution should be used within the hour and then discarded.
3. Add 20 MBq ^{125}I to an empty tube (annotated “the mixing tube”).
4. Add 5 µl 0.1% Acetic acid to the mixing tube.
5. Add 2.5 µl of the SPMB solution to the mixing tube.
6. Add 5 µl of the CAT solution to the mixing tube. Mix properly and incubate at room temperature for 5 minutes.

7. Add 8 µl of the Na_2SO_5 solution to the mixing tube and resuspend carefully.
8. Add the protein to the mixing tube.
9. Add 200 µl of the borate buffer to the mixing tube. Additional borate buffer may be added if the pH of the solution is clearly below pH 9.
10. Incubate for 30-45 minutes at room temperature.
11. Remove excess ^{125}I using a gel filtration column, such as a Sephadex G-25 column or equivalent matrix, in PBS. Example, with a NAP-5 column:
    a. Equilibrate column with PBS according to instructions from the manufacturer.
    b. Add the ^{125}I-labeled protein solution to the column together with additional PBS to get a total sample volume of 500 µl.
    c. Eluate with 1 ml PBS.
12. Measure the activity from the background (Bg), the mixing tube (Mix), the column (Cn), and the tube with the eluted labeled protein solution (Elu) and calculate the yield:

$$Yield = \frac{Act_{Elu} - Act_{Bg}}{(Act_{Mix} - Act_{Bg}) + (Act_{Cn} - Act_{Bg}) + (Act_{Elu} - Act_{Bg})}$$

13. Store the conjugate under the same conditions as the unlabeled protein (protein dependent). Siliconized tubes may be used to reduce the risk of non-specific binding during storage.