Direct labeling of protein with ^{125}I

This protocol describes how proteins can be labeled with ^{125}I, suitable for measurements 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 tyrosine residues and may affect the binding properties of some proteins.

Materials

- Protein (preferably at least 0.5 mg/ml in stock solution):
  - -For antibodies or proteins of ~150 kDa: 20-100 µg
  - -For other molecular weights: Aim at a final concentration of 100-600 nM in 1 ml labeled solution
- ^{125}I
- Chloramine-T (CAT)
- Na_2SO_5
- Gel filtration column, e.g. NAP™-5
- PBS
- Ice

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. Add 5-20 MBq ^{125}I to an empty tube (annotated “the mixing tube”).
3. Add the protein and 120 µl of PBS to the mixing tube.
4. Add 20 µl of the CAT solution and mix properly. This will start the reaction. Immediately put the mixing tube on ice and incubate for 5 minutes.
5. Add 40 µl of the Na_2SO_5 solution and mix to stop the reaction.
6. 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.

7. 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:

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

8. 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.