Iodination and Injection of ^{125}I-Protein

I. Column Preparation (Sephadex G-25 Column)

1. Swell resin with H2O
2. Pack into a 10ml-column (10ml pipet)
3. Run the column with 1%BSA in PBS

II. Chloramine T Method

Protein amount: ~100ug in PBS (100-200ul volume)

1. Add ^{125}I (2-5ul) 0.2~0.5mCi / protein and stand 1 min. at RT
2. Add 2.5ul of Chloramine T (1.6mmol) and stand 1 min. at RT
3. Add 5ul of sodium metabisulfite (4mmol) and stand 1 min. at RT

III. Isolation of ^{125}I-Labeled Protein

1. Load onto the Sephadex G-25 column and collect 1ml factions (about 20 tubes)
2. Count off 5ul of each sample and proceed to TCA precipitation with three samples which have the high cpm.

IV. TCA Precipitation

1. Add 5ul of the collected fraction to tube and add 0.5ml of 2.5% BSA and 0.5 ml of 10% TCA
2. Stand 10min. at 4°C and spin down at 4°C for 5 min. at 2768 rpm
3. Separate pellet and supernatant and count them

$$\text{TCA ppt count: } \frac{\text{cpm of pellet}}{\text{Cpm of sup + cpm of pellet}} \times 100$$

TCA ppt count should be over 95%

V. Injection dose to a mouse

- ^{125}I-labeled protein should be injected within a day
- About 20ug protein/mouse or 5-30 x 10^6 cpm/mouse

1. Take blood sample (50ul) from the mouse at 5 min, 15 min, 30min, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours.
2. 5ul aliquots of sample will be precipitated with TCA and calculate the TCA precipitable count.

SOLUTIONS

1. 0.05M PBW

NaH_2PO_4 (mw 137.99) – 0.069 g in 10 ml H2O
Adjust to pH7.4

2. Sol. Metabisulfite Solution

Stock solution: Sodium metabisulfita 4.8mg in 1ml H2O

Dilution prior to use: Stock Solution : H_2O = 1 : 50
(10 ul stock + 490 ul H_2O)

3. 0.4M PBW
   $NaHPO_4$ 0.55g in 10ml H_2O
   Adjust to pH 7.4

4. 0.01M PBS : Elution buffer

$NaHPO_4$	(mw 137.99)	0.69 g
NaCl	(mw 58.44)	4.38 g
BSA (0.1%)		0.5 g

   All that in 500ml
   Adjust pH to 7.4

5. 10% TCA Solution

   TCA 5g in 50ml H_2O

6. 2.5% BSA Solution

   BSA 0.25g in 10ml H_2O

7. Chloramine T Solution
   Stock solution: Chloramine T 10mg in 1ml of 0.05M PBW

   Dilution before use: 10ul of stock solution in 730ul of PBS