Procedure 6: To prepare Biotinylated Antibody Conjugate.

Reagents:
Purified IgG (3 mg/ml)
Sodium Hydrogen Carbonate
($NaHCO_3$)
N-Hydroxy-Succinimidobiotin
(NHS-d-biotin)
Phosphate Buffered Saline (PBS)
Dimethyl Sulfoxide (DMSO)
Deionised water

Materials & Equipment:

Analytical Balance
pH Meter (calibrated 7 - 10)
Beakers and graduated cylinders
Magnetic stirrer
Dialysis tubing
Spectrophotometer

Reagent Preparation:

Preparation of 0.1M $NaHCO_3$ (500ml)

1. Record component batch number(s).

2. Dissolve 4.2g $NaHCO_3$ in 500ml deionised water and check pH 8.3.

3. Dispense 50ml into a container. Pour remaining solution into a reagent bottle.

Preparation of Phosphate Buffered Saline (PBS):

1. Record component batch number(s).

2. Dissolve 16 PBS tablets in 1600ml deionised water.

3. Cool buffer to 4^{o}C overnight.

Preparation of 29.3mM N-Hydroxy-Succinimidobiotin / DMSO

1. Dissolve 5mg NHS-d-biotin in 500µl DMSO.

2. Use immediately.

Procedure:

Day 1

1. Cut a suitable length of dialysis tubing for 4 ml purified IgG (3 mg/ml).

2. Presoak dialysis tubing in deionised water for at least 5 minutes before use.

3. Dialyse 4ml of IgG (3 mg/ml) against 2 changes of 200ml 0.1M $NaHCO_3$ (with stirring) overnight at 4^oC.

Day 2

4. Remove IgG from dialysis.

5. Measure the antibody concentration of the solution spectrophotometrically at 280nm within the absorbance range of 0.2 - 1.5. Dilute an aliquot of the sample to facilitate A_{280nm} measurement. [IgG] mg/ml = A_{280nm} /1.38.

6. Adjust antibody concentration to 2.9mg/ml with the 0.1M $NaHCO_3$ if required.

7. Add 1 volume NHS-d-biotin/DMSO dropwise to 9 volumes of the IgG solution i.e., add 400µl of NHS-d-biotin/DMSO to 3.6ml of IgG (2.9mg/ml)**.**

8. Allow the reaction to occur at room temperature (22^0C) for 1 hour with continuous mixing. Note ambient temperature.

9. Transfer the reaction mixture to dialysis tubing (presoaked in deionised water for 5 minutes).

10. Dialyse the reaction mixture against 4 changes of 400ml chilled PBS over 48 hours.

11. Measure the antibody concentration of the solution spectrophotometrically at 280nm within the absorbance range 0.2 - 1.5. Dilute an aliquot of the sample to facilitate A_{280nm} measurement.

Day 4

12. Transfer the dialysed and biotinylated IgG to a storage container.

13. Store at 4^oC for up to 3 days. For longterm storage aliquot 500µl into eppendorfs and store at -20^oC until required.