Alum Precipitation

$AlK(SO_4)_2 \cdot 12H_2O$

1. Mix 1 volume of 2% protein to be precipitated and 9 volumes of 9% $KAL(SO_4)_2 \cdot 12H_2O$ (9g + 100 ml 1X PBS)
2. Add 5N NaOH dropwise to pH 6.5 - use pH paper (see white precipitate).
3. Allow to stand for 2-24 hrs (usually 3 hrs) at 4°C.
4. Spin down precipitate at ~ 3000 rpm in Sorvall (small head)–don't pellet too tightly
5. Wash pellet 3X with 1X PBS (using large volumes)–spin at 3,000 rpm (~1000g)
6. Resuspend pellet in known volume of 1X PBS - cannot do ODs (must estimate protein concentration from input assuming no loss)
7. Store at 4°C at 5-10 mgs/ml
8. Add merthiolate to 1/50,000 - (Sigma Thimerosal T-5125 [redacted])
   make 0.5% merthiolate stock in PBS.
   Then dilute 1:250 in alum suspension

May be injected for sensitization i.p. or i.v. in low concentrations

Alum precipitation enhances immunogenecity of proteins.

N.B.

When protein concentration is less than 2% (i.e. down to about 5mg/ml) alum should be added on basis of amount of protein rather than on volume of protein solution eg If precipitating 10mg of protein at 5mg/ml add 4mL of protein + 9mL of 9% alum solution

2% protein = 2gm/100ml or 20mg/ml

9% alum = 9gm/100 or 90mg/ml

9:1 ratio

9X90 : 1X20

810 : 20 or 40.5mg alum : 1mg protein