The University of Georgia

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STANDARD OPERATING PROCEDURES

Title: *Ammonium Sulfate Precipitation of Antibodies*

No: RTLP-GL-Ab-2

Location:	Approval Date:	Supersedes Date:
Old CCRC Tripp Lab	10 September 2004	
Materials:		Page 1 of 1

- Lab coat
- Gloves
- Timer
- Aspirator
- Transfer Pipette
- 15ml tubes
- 4° C centrifuge
- Ice bucket w/ ice
- 4° C cold storage
- 4° C centrifuge
- 4° C cold storage
- distilled H_2O
- Microwave
- Pipettes
- Pipetteman
- Pipette aid
- Pipetteman tips
- 50ml tubes
- Saturated ammonium sulfate (SAS)
- Phosphate buffered saline (PBS)
- Dialysis tubing or cassettes
- Spectrophotometer
- Borate Buffer*

*Dependent upon whether product will be used as is or run on protein G column

Procedure:

1. Centrifuge monoclonal antibody supernatant for 30 minutes at 20,000 x g, 4°C. When centrifugation is complete, save the supernatant and store on ice.
2. Prepare saturated ammonium sulfate (SAS) by dissolving 760 grams of ammonium sulfate in one liter of boiling distilled water.
3. Let SAS cool and store at 4° C.
4. Keeping the SAS and supernatant on ice, add SAS drop-wise to the medium while stirring to 45% (vol/vol). Leave 2h at 4°C or overnight in cold room.
5. Centrifuge the mixture at 3000 x g for 30 minutes at 4°C, decant the supernatant and save precipitate (pellet).
6. Dissolve the precipitate in PBS or borate buffer*.
   NOTE: The buffer you choose depends on whether it will be used as is, or run through a protein G column.
7. Dialyze against >20 volumes of buffer chosen for 24-48h at 4°C making sure to change the buffer at least 4 times during dialysis.
8. Determine the IgG concentration using A_{280}.