

## IgY Purification Record

Name of Operator: \_\_\_\_\_ Date: \_\_\_\_\_ Chicken No.: \_\_\_\_\_

Immunizing Antigen: \_\_\_\_\_ Lot size (# eggs): \_\_\_\_\_ Date of Eggs: \_\_\_\_\_

Yield IgY per ml egg yolk (mg) \_\_\_\_\_ IgY Concentration: \_\_\_\_\_ mg/ml

Total IgY Yield: \_\_\_\_\_ Total vol. (ml) \_\_\_\_\_

### Instructions

1. Assume that 1 gram of yolk is equal to 1 ml.
  - \* volume of egg yolks = \_\_\_\_\_ ml (vol. A)
2. Add 5 times the egg yolk volume (A) of cold Reagent A to the container and stir gently:
  - \* volume of Reagent A =  $5 \times (A) =$  \_\_\_\_\_ ml (vol. B)
  - \* lot no. Reagent A: \_\_\_\_\_
3. Place in fridge (at least 2 hours — up to overnight).
  - \* volume of Reagent A =  $5 \times (A) =$  \_\_\_\_\_ ml (vol. B)
  - \* lot no. Reagent A: \_\_\_\_\_
4. Centrifuge solution at  $10,000 \times g$  ( 4 degrees C) for 15 min. Discard lipidy pellet. Collect supernatant into graduated cylinder by filtering through gauze inserted into filter paper. The supernatant should be colourless and translucent. If particulates are present, centrifugation step should be repeated. Measure volume of supt.
  - \* volume of supernatant (ml) = \_\_\_\_\_ (vol. C)
  - \* centrifugation step repeated? \_\_\_\_\_ yes \_\_\_\_\_ no
  - \* colourless, translucent supt? \_\_\_\_\_ yes \_\_\_\_\_ no
5. To supernatant (vol. C), add the same volume of cold Reagent B and mix gently. Let sit for at least 1 hr in the fridge (up to overnight).
  - \* volume Reagent B added (vol. C): \_\_\_\_\_ ml
  - \* lot no. Reagent B: \_\_\_\_\_
  - \* incubation time: \_\_\_\_\_
6. Centrifuge 4 degrees C at  $10,000 \times g$  for 15 min. Resuspend the pellet in the original egg yolk volume (vol. A) with PBS:
  - \* final IgY volume (vol. A): \_\_\_\_\_ ml
7. To determine the IgY conc. and yield, prepare two 1/20 dilutions of the purified IgY in PBS (ie add 50 ul of IgY to 950 ul PBS). Measure the absorbance at 280 nm using PBS as blank.
  - \* Abs#1= \_\_\_\_\_ Abs#2= \_\_\_\_\_ Avg. Abs = \_\_\_\_\_
  - \* concentration =  $(\text{avg Abs} \times 20)/1.35 =$  \_\_\_\_\_ mg/ml
  - \* yield = concentration x final
  - \* volume (vol. A) = \_\_\_\_\_ mg
8. Filter sterilize or add a preservative and refrigerate. IgY activity will remain unchanged for at least one year.
  - \* Filter sterilize? \_\_\_\_\_ yes \_\_\_\_\_ no
  - \* Preservative? \_\_\_\_\_ yes \_\_\_\_\_ no
  - \* If yes, what kind? \_\_\_\_\_ Concentration: \_\_\_\_\_