

# 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 (mg) \_\_\_\_\_ 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:

**Vol of Reagent A = 5 x (A) = \_\_\_\_\_ ml Lot no. Reagent A: \_\_\_\_\_**

3. Place in fridge (at least 2 hours — up to overnight):

**Incubation Time = \_\_\_\_\_ hr**

4. Centrifuge solution at 2,000 x g ( 4°C) for 15 min. Collect supernatant into graduated cylinder and discard lipid pellet. by filtering through gauze inserted into filter paper. The supernatant should be colourless and translucent. If particulates are present, filter through cotton gauze or filter paper. Measure volume of supernatant.

**Vol. of supernatant (ml) = \_\_\_\_\_ (vol. B)**

**Colourless, translucent supernatant? \_\_\_\_\_ yes \_\_\_\_\_ no**

5. To supernatant (vol. B), add the same volume of cold Reagent B and mix gently. Let sit for at least 1 hr in the fridge (up to overnight).

**Vol. Reagent B added (vol. C): \_\_\_\_\_ ml Lot no. Reagent B: \_\_\_\_\_**

**Incubation time: \_\_\_\_\_ hr**

6. Centrifuge 4°C at 2,000 x 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 1/10 dilutions of the purified IgY in PBS (ie add 100 ul of IgY to 900 ul PBS). Measure the absorbance at 280 nm using PBS as blank.

**Abs#1= \_\_\_\_\_ Abs#2= \_\_\_\_\_ Avg. Abs = \_\_\_\_\_**

**Concentration = (Avg Abs x 10)/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 sterilized? \_\_\_\_ yes \_\_\_\_ no    Preservative? \_\_\_\_ yes \_\_\_\_ no

If yes, what kind? \_\_\_\_\_    Concentration: \_\_\_\_\_