

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: _____