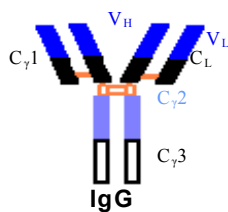


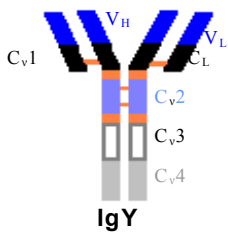
EggsPure IgY

A 60 min-protocol IgY PURIFICATION KIT

EN



IgG



IgY

IgG and IgY structure comparison

Introduction

Chickens are excellent producers of antibodies and the intensity of their immune response is comparable to mammals, but has the advantage of being faster. Producing antibodies in chicken eggs is an alternative to animal experimentation (Concept of three R's, Replacement, Reduction and Refinement, developed by Russel and Burch, 1969). IgY are obtained from egg yolk with no need for bleeding the animals.

Every day, the hen lay an egg with 50 to 100 mg of total IgY.

IgY presents some major features:

- IgY does not bind bacterial or mammalian Fc receptors,
- IgY does not activate mammalian complement system.

IgY behaves like natural F(ab')₂ analogue but presents a larger Fc fragment. Using chicken IgY avoids interference due to anti-mammalian IgG antibodies.

The lipids which are present in abundance in egg yolk and the fact that chicken IgY does not bind to protein A and G has made the purification of IgY somehow complicated.

The *EggsPure IgY* kit provides a quick, simple and efficient method to extract IgY from egg yolk.

Using *EggsPure IgY*, total IgY yields around 50 mg with a purity of at least 90%.

IgY thus purified are suitable for direct use in ELISA, immunoblotting, flow cytometry, immunochromatography and affinity purification methods. From the total IgY extracted by the *EggsPure IgY*, specific IgY antibodies are then purified from by affinity chromatography using specific antigen immobilized in a chromatography column. Specific IgY antibodies represent 2 to 10 % of total IgY antibodies.

Kit contents

1. Kit contents for 6 eggs isolations (100 mL of yolk):

EggsPure IgY (6)

LT01110

- Reagent A : 420 mL
- Reagent B : 380 mL
- Reagent C : 265 mL
- Agro-Bio egg separator
- 1 fast-flow filter paper
- 2 plastic hooks

- 2 fast-flow filter paper
- 2 plastic hooks

Additional utensil necessary, but not provided:

- Phosphate Buffered Saline (PBS 1X)
- Centrifuge and centrifuge tubes
- Funnel
- Magnetic stir bar and stir plate
- Spatula
- Graduated cylinders

2. Kit contents for 12 eggs isolations (200 mL of yolk):

EggsPure IgY (12)

LT01111

- Reagent A : 2 x 420 mL
- Reagent B : 2 x 380 mL
- Reagent C : 2 x 265 mL
- Agro-Bio egg separator

Instructions



Egg yolk separation

Note :
Up to 6 yolks (100 mL of egg yolk) may be extracted with the LTO1110 kit and 12 eggs (200 mL of egg yolk) with the LTO1111 kit.

Note :
Eggs can be stored at 2-8 °C before IgY extraction.

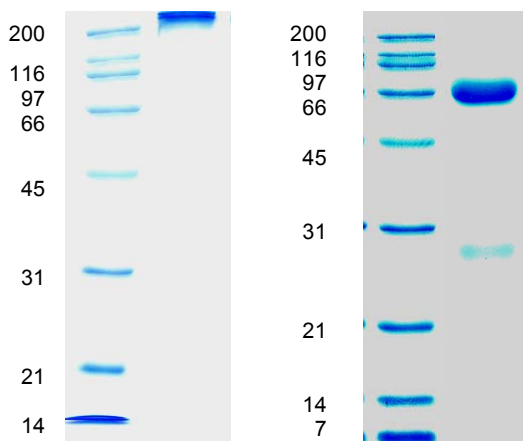


Yolk membrane elimination

Note :
This protocol will also work with duck eggs.

Note :
A data recording papersheet is provided in the kit to facilitate the volume calculations for solutions A, B and C according to the number of eggs extracted.

A computer datasheet can also be obtained by contacting at +33.2.38.64.83.50



Electrophoresis of IgY purify with EggsPure IgY :

Left : Total IgY at 200 kDa

Right : Heavy chain at 60-70 kDa and light chain at 25 kDa

1. Egg yolk separation

Eliminate the white egg using the egg separator. The yolk membrane is eliminated too using plastic hooks and the yolk volume is measured. (Keep the eggs at room temperature* for 30 minutes before use in order to facilitate the separation of white).

Record the volume of the egg yolk (V1) using a graduated tube rinsed with PBS.

2. Delipidation step

Add 4 volumes (4 x V1 mL) of reagent A to the yolks and stir until homogenisation. Keep on stirring for 5 minutes at room temperature. The mixture is then transferred into a centrifuge tube.

Centrifuge at 2000xg for 10 minutes, at room temperature.

Filter the supernatant through fast-flow filter paper and collect it into a graduated cylinder (1L). Record the volume of supernatant.

3. Protein precipitation

Dilute the supernatant by adding an equal volume of reagent B while agitating with a magnetic stir bar.

Keep on stirring for 5 minutes at room temperature.

Centrifuge at 2000xg for 10 minutes at room temperature.

4. IgY Extraction

Discard the supernatant and dissolve the precipitate in 2.5 x V1 volume of reagent C. (To resuspend the pellet, scrape with a spatula and gently vortex. Pay attention that the pellet is translucent so it could be difficult to observe it).

The mixture is stirred for 5 minutes at room temperature

Centrifuge at 2000xg for 10 minutes at room temperature and discard the supernatant.

The precipitate is then diluted in a volume of PBS 1X equal to 0.5 x egg yolk volume (V1) (The IgY concentration will be approximately 5 mg/mL and 90% pure).

* Room temperature = RT = 18°-25 °C

5. IgY concentration

Concentration may be determined from the absorbance at 280 nm of Extract IgY diluted at 1:10.

$$\text{IgY concentration} = A(280) \times 10 \times 0.74 \text{ mg/mL}$$