

Pierce™ Chicken IgY Purification Kit

44918 44922

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Number	Description
44918	Pierce Chicken IgY Purification Kit , sufficient reagents to purify IgY from 5 egg yolks Kit Contents: Delipidation Reagent , 500mL (available separately as Product No. 21055) IgY Precipitation Reagent , 500mL (available separately as Product No. 21057) Egg Separator , 1 unit
44922	Pierce Chicken IgY Purification Kit , same as Product No. 44918 but with 5 units of each component, sufficient to purify IgY from 25 egg yolks

Storage: Upon receipt store kit at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Chicken IgY Purification Kit provides a robust, quick and simple method to purify IgY from egg yolks. Although a large amount of IgY is produced in eggs, purification of IgY has been problematic because of the large amount of lipid that is present in egg yolk and because IgY does not bind to either IgG-binding proteins like Protein A or Protein G. The IgY Purification Kit simplifies the otherwise difficult, time-consuming and multi-step protocols of conventional IgY purification methods.¹⁻³ With this kit, 80-120mg of 90% pure IgY can be easily obtained from each egg yolk. An optional second precipitation will increase the IgY purity (e.g., single band by SDS-PAGE with 4µg/lane and coomassie staining) without substantially sacrificing yield. IgY purified with this kit is suitable for direct use in ELISA, Western blotting and affinity purification methods. Antigen-specific IgY can be purified from the total IgY by affinity to the immobilized specific antigen.

The eggs of immunized chickens are an economical and abundant source of polyclonal antibodies. A single egg may contain as much antibody as can be obtained from an average bleed of a rabbit. Immunogen-specific antibody generally is from 1-10% of the total IgY in each egg. The eggs from immunized chickens offer a nearly continual daily source of milligram quantities of specific polyclonal antibody. When using chickens, there is a higher potential of producing antibodies to mammalian antigens than when using rabbits or goats. In addition, chicken IgY does not cross-react with mammalian IgG and does not bind bacterial or mammalian Fc receptors. It has been reported, that nonspecific binding is reduced and the need to do cross-species immunoabsorptions is eliminated when using chicken antibodies.^{4,5}

Important Product Information

- If eggs will be collected over several weeks or months for final processing in one batch, separate and partially extract the egg yolks as they are collected (see note after Step A.6 of the procedure) and store them frozen.
- Ensure that reagents and eggs are cold (refrigerated) before beginning the IgY purification procedure.
- The kit is intended for use with egg yolks that have been refrigerated for less than two months. For older eggs, use six volumes of Delipidation Reagent in step B.1 (instead of five times as indicated in the procedure) and incubate in the refrigerator for at least 24 hours before centrifugation. Results with old yolks are unpredictable.
- A purification record sheet is attached as the last page of these instructions. Make photocopies as needed.

Additional Materials Required

- Egg(s) collected from immunized chickens
- Phosphate-buffered saline (PBS): 100mM sodium phosphate, 150mM sodium chloride; pH 7.2 (e.g., Product No. 28372). For long-term storage, sterile-filter the PBS and store refrigerated. If microbial growth occurs, discard the PBS.
- Ultrapure (distilled or deionized) water
- Paper towels
- Balance
- Refrigerated, high-speed centrifuge and centrifuge tubes that will hold at least 150mL
- Magnetic stir bar and stir plate
- Beakers, graduated cylinders and glass Pasteur pipettes

IgY Purification Procedure

A. Separation and Isolation of Egg Yolk

1. Determine and record the tare weight of an empty clean glass beaker. Beaker capacity must be > 125mL per egg yolk.
2. Separate the cold egg yolk from the egg white using the Egg Separator.
3. When most of the egg white has drained from the yolk, rinse the yolk sac with ultrapure water.
4. Carefully roll the egg sac onto a clean, dry paper towel. Roll the intact egg sac around on the paper towel to remove adhering egg white. Then position the egg sac near the edge of one side of the paper towel.
5. Suspend the egg sac in the paper towel over the tared beaker. Then puncture the egg sac with the Pasteur pipette and collect as much of the egg yolk as possible in the tared beaker. Allow sufficient time for the egg sac to drain completely. Discard the paper towel and the empty egg sac.
6. Re-weigh the beaker and record the gram weight of the egg yolk as the yolk volume in milliliters. For this procedure, assume that 1 milliliter of egg yolk weighs 1 gram.

Note: To store separated egg yolks for later use, slowly add two times the original yolk volume of cold Delipidation Reagent to the egg yolk while stirring gently and continuously with a magnetic stir bar. Continue gentle stirring until the egg yolk and the Delipidation Reagent are well mixed. Store mixture at -20°C. Egg yolk samples may be frozen for about one year when prepared in this manner.

B. Delipidation of Yolk Solution

1. With slow and gentle continuous mixing, add five times the egg yolk volume of cold Delipidation Reagent to the egg yolk. Continue gentle stirring until the egg yolk and the Delipidation Reagent are mixed well.

Note: For yolks that had been mixed and frozen in two volumes of Delipidation Reagent (see note after Step A.6), thaw the sample and add three (instead of five) yolk-volumes of Delipidation Reagent while gently mixing. It is normal for this mixture to have a curdled appearance.

2. Cover and incubate the beaker of diluted egg yolk at 4°C for 2-24 hours.
3. Gently remix the diluted egg yolk solution before adding it to a centrifuge bottle or tube. Centrifuge for 15 minutes at 4000-10,000 × g in a refrigerated centrifuge.
4. Decant the supernatant, which should be colorless and translucent, into a graduated cylinder and record the volume.

Notes:

- If the supernatant contains particulate matter but is otherwise translucent and colorless, remove the particulate material by filtering the supernatant through fast-flow filter paper.
- If the supernatant is yellow and viscous or the pellet is not solid, try centrifuging again for a longer time. If the results are the same, add an additional yolk-volume of cold Delipidation Reagent, mix well, incubate overnight at 4°C, and centrifuge as before. The pellet should be more solid. The supernatant may be slightly yellow and somewhat opaque, but not viscous.

IgY Precipitation

1. Pour supernatant into a clean beaker. While stirring gently, add an equal volume of cold IgY Precipitation Reagent. Continue gentle mixing for two minutes.
2. Cover beaker and incubate suspension at 4°C for 1 hour to overnight.
3. Gently remix the suspension and then add it to a cold centrifuge bottle or tube. Centrifuge for 15 minutes at 4000-10,000 × g in a refrigerated centrifuge. Discard the supernatant.
4. **Note:** Presence of a diffuse white precipitate but no visible white pellet indicates that the supernatant was not adequately delipidated following centrifugation with the Delipidation Reagent. This can occur with older egg yolks (those older than 2 months) or with frozen eggs that were not stored in Delipidation Reagent. In this case, it is best to start with another egg, this time using a volume of cold Delipidation Reagent equal to six times the volume of the egg yolks and, after mixing, incubate the delipidation mixture overnight at 4°C.
5. To the retained pellet, which is the purified IgY, add a volume of PBS equal to the original volume of the egg yolk and mix gently until it is completely dissolved. Measure and record the volume of the purified IgY solution.

Note: To obtain more pure IgY with only a small decline in yield, repeat the precipitation steps (Section C). Before deciding to perform a second round of precipitation, consider estimating the IgY purity by SDS-PAGE (see Additional Information, Section A) or by testing the IgY in the intended application. These results may indicate that a second round of precipitation is not necessary.

Additional Information

A. Determine IgY Concentration, Yield Per Yolk, and Purity

Prepare duplicate 20-fold dilutions of the purified IgY in PBS (e.g., 50µL of purified IgY + 950µL of PBS). Measure the absorbance at 280nm (A_{280}) in a 1cm pathlength quartz cuvette. Calculate the IgY concentration (mg/mL) based on the following relationship: A_{280} divided by 1.4 equals concentration in mg/ml. Multiply by 20 to obtain the concentration of the undiluted IgY sample. Typically, the entire purification procedure results in an IgY solution that is 4-7mg/mL.

Calculate the total amount of purified IgY obtained by multiplying the IgY concentration by the volume of the purified IgY solution. Because egg yolk sizes vary greatly, it is often most meaningful to express the purified IgY yield as the milligrams of IgY obtained per milliliter of egg yolk. To store the purified IgY, filter-sterilize the solution and/or add a preservative.

Estimate purity by preparing 2-4µg IgY samples in SDS-PAGE sample loading buffer and loading them in an appropriate gel (e.g., 4-20% Thermo Scientific Precise Protein Gel, Product No. 25204). After electrophoresis, stain the gel with a suitable protein stain (e.g., Thermo Scientific Imperial Protein Stain, Product No. 24615; Thermo Scientific Pierce Silver Stain, Product No. 24612). To estimate percent purity, compare IgY band intensities (densitometry) to the total density of other bands. The mass of nonreduced IgY is 180kDa; in a reducing gel, IgY migrates as 22 and 68kDa bands that correspond to light and heavy chains, respectively.

B. Immunization Protocol for Chickens

The following is an example immunization protocol that has been used successfully to generate polyclonal antibodies in chickens. White leghorn chickens are frequently used because they produce large eggs. Individual hens will respond differently to immunization with a particular antigen. Antibody titers vary, as will their stability.

1. Collect two pre-immune eggs before performing any immunizations.
2. On day 0, inject between 0.02 and 0.5mg of antigen with Freund's Complete Adjuvant (Product No. 77140) subcutaneously and/or intramuscularly into the breast tissue of the hen at multiple sites. Use a total antigen/adjuvant volume of 1mL, with the adjuvant comprising one-half and two-thirds of the volume. (Generally, chickens require the same amount of antigen as rabbits for an immunization.)
3. Repeat immunizations on days 10, 20 and 30 using Freund's Incomplete Adjuvant (Product No. 77145) and about half the amount of antigen. Specific antibody may be detected by day 30 in the eggs. For prolonged antibody production, continue to boost hens every two months.

Cited References

1. Jensenius, J.C., *et al.* (1981). Eggs: conveniently packaged antibodies. Methods for purification of yolk IgG. *J Immunol Methods* **46**:63.
2. Polson, A., *et al.* (1985). Improvements in the isolation of IgY from the yolks of eggs laid by immunized hens. *Immunol Invest* **14**:323.
3. Akita, E.M. and Nakai, S. (1993). Comparison of four purification methods for the production of immunoglobulins from eggs laid by hens immunized with an enterotoxigenic *E. coli* strain. *J Immunol Methods* **160**:207.
4. Larsson, A. and Sjoquist, J. (1990). Chicken IgY: Utilizing the evolutionary difference. *Comp Immun Microbiol Infect* **13**:199.
5. Larsson, A., *et al.* (1993). Chicken antibodies: taking advantage of evolution – a review. *Poultry Sci* **72**:1807.

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Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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IgY Purification Record

Final Data

Name of operator:	Lot size (# of eggs):	Final IgY concentration:
Date:	Date of eggs:	IgY yield per ml of yolk:
Chicken ID or #:	Total yolk volume:	Total IgY yield:
Immunizing Antigen:	Final IgY volume in PBS:	Purity of IgY:

Procedure Data

A. Separation and Isolation of Egg Yolk

Separate yolk from egg white, clean it on a paper towel, rupture and drain into pre-weighed beaker:

Gross weight (beaker + yolks) = _____ g
 Tare weight (beaker) = _____ g
 Net weight of yolks = _____ g
 Volume of egg yolks = _____ ml (Vol. A)

B. Delipidation of Yolk Solution

Add five volumes of cold Delipidation Reagent, mix and incubate Incubate at 4°C for 2-24 hours:

Volume of Delipidation Reagent used (5 × Vol. A) = _____ ml (Vol. B)
 Lot no. of the Delipidation Reagent used = _____
 Incubation: Start date and time = _____ End date and time: _____

Centrifuge solution at 10,000 × g for 15 minutes (at 4 deg. C):

Centrifugation: Speed = _____ Time = _____ Temperature = _____

Decant supernatant into a graduated cylinder and discard the lipid-containing pellet:

Volume of supernatant = _____ ml (Vol. C)
 Appearance of the supernatant: _____

C. IgY Precipitation

Add equal volume of cold Precipitation Reagent, mix gently and incubate at 4°C for 1 hour to overnight.

Volume of Precipitation Reagent used (1 × Vol. C) = _____ ml (Vol. D)
 Lot no. of the Precipitation Reagent used = _____
 Incubation: Start date and time = _____ End date and time: _____

Centrifuge at 10,000 × g for 15 minutes (at 4°C). Discard the supernatant. Dissolve the pellet in a volume of PBS equal to the original volume of the yolks (Vol. A). Mix gently until the IgY is completely dissolved.

Centrifugation: Speed = _____ Time = _____ Temperature = _____
 Final volume of the purified IgY in PBS: = _____ ml (Vol. E)

D. Determine Yield

Determine the IgY concentration by diluting (in duplicate) the purified IgY 1: 20 in PBS (50µL of IgY + 950µL PBS) and measuring the absorbance at 280nm using PBS in the reference cuvette.

$A_{280} \#1 =$ _____ $A_{280} \#2 =$ _____ Avg. $A_{280} =$ _____
 IgY concentration = $[Avg. A_{280} \times 20] / 1.4 =$ _____ mg/ml
 IgY yield = IgY concentration × final IgY solution volume (Vol. E) = _____ mg total IgY

E. Prepare for storage

Filter sterilize or add a preservative to the purified IgY solution and store refrigerated.

Filter-sterilized? Yes/No Manufacturer: _____ Cat. #: _____ Lot #: _____ Pore size: _____
 Preservative added? Yes/No Type: _____ Final concentration: _____
 Manufacturer: _____ Cat. #: _____ Lot #: _____

F. Assess Purity by SDS-PAGE

Approximate purity based on relative staining intensity of IgY bands in gel: _____