



Promega

Technical Bulletin

EGGstract® IgY Purification System

INSTRUCTIONS FOR USE OF PRODUCTS G2610 AND G1531.



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EGGstract[®] IgY Purification System

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1. Description.....	1
2. Product Components and Storage Conditions	2
3. EGGstract [®] IgY Purification System Protocol	3
A. IgY Purification Protocol	3
B. Analysis of IgY Concentration and Purity	4
4. Composition of Buffers and Solutions	5
5. Related Products	5
6. References	6
7. EGGStract [®] IgY Purification System Batch Record.....	6

1. Description

The EGGstract[®] IgY Purification System provides a quick and easy method for the isolation of egg yolk immunoglobulin (IgY), the ~180kDa chicken IgG homolog, from chicken egg yolks. The yolk of eggs laid by immunized chickens has been recognized as an excellent source of polyclonal antibodies (pAb) (1). Because a single egg contains as much antibody as an average bleed from a rabbit, this simple, noninvasive approach represents an appealing alternative to the generation of pAb in rabbits. IgY purification does not require animal bleeding, and the eggs of immunized chickens provide a continual, daily source of pAb. Additionally, IgY to certain biological antigens that fail to elicit a humoral immune response in rabbits or other mammals due to species relatedness can be produced in chickens (2).

The large amount of lipid present in egg yolk and the failure of IgY to bind to Protein A and Protein G have previously presented problems in the purification of chicken IgY. Several methods have been described for the purification of IgY from egg yolks (3-5); however, these protocols consist of numerous time-consuming steps, including salting in, chromatography and dialysis. In contrast, the EGGstract[®] IgY Purification System is a rapid and highly effective IgY purification method.

To purify IgY using the EGGstract® System, simply separate the yolks from immunized chicken eggs and dilute the yolks into Precipitation Solution A. After mixing, remove the lipids by centrifugation and precipitate the IgY with Precipitation Solution B. The entire purification procedure can be performed in one hour, with roughly 30 minutes of hands-on time. Using this system, yields typically range between 40–80mg of approximately 75% pure IgY per egg (Figure 1). An optional second precipitation with Precipitation Solution B can increase IgY purity to approximately 90% (Figure 1).

IgY purified using the EGGstract® System is suitable for immunoblotting (2), ELISA (6), immunostaining and other standard immunological procedures (1).

2. Product Components and Storage Conditions

Product	Size	Cat.#
EGGstract® IgY Purification System	6 isolations	G2610

Each system contains sufficient reagents and components for the rapid isolation of polyclonal IgY from 6 eggs of immunized chickens. Includes:

- 1 Egg Separator
- 3 Sterile Gauze Packets
- 455ml Precipitation Solution A
- 255ml Precipitation Solution B

Product	Size	Cat.#
EGGstract® IgY Purification System	25 isolations	G1531

Each system contains sufficient reagents and components for the rapid isolation of polyclonal IgY from 25 eggs of immunized chickens. Includes:

- 1 Egg Separator
- 6 Sterile Gauze Packets
- 1,960ml Precipitation Solution A (2 × 980ml)
- 1,040ml Precipitation Solution B

Note: Up to six yolks (approximately 15–25ml each) from the eggs of an immunized white leghorn chicken may be processed simultaneously with a single system (Cat.# G2610); the larger system (Cat.# G1531) may be used to process up to 25 yolks.

Storage Conditions: Store all reagents and components of the EGGstract® IgY Purification System at room temperature, where they are stable for one year from the date of purchase.

3. EGGstract® IgY Purification System Protocol

The EGGstract® IgY Purification System yields high amounts of biologically active polyclonal antibody (typically 2–5mg of IgY per milliliter of yolk) in one hour at room temperature with approximately 75% purity of the end product. An EGGstract® IgY Purification System Batch Record form is provided in Section 7 for notebook documentation of each antibody isolation. **Note:** Purity can be increased to approximately 90% by performing a second precipitation with Precipitation Solution B; this optional, second precipitation requires an additional 15 minutes.

Note: This procedure will also work with pheasant or turkey eggs.

3.A. IgY Purification Protocol

Materials to Be Supplied by the User

(Solution compositions are provided in Section 4.)

- Eggs from immunized chicken(s)
(See reference 1 for an immunization strategy.)
- Phosphate-buffered saline (PBS)
- **Optional:** IgY storage buffer

Note: Eggs can be stored at 4°C for up to one year prior to IgY purification.

1. Allow the egg(s) to warm to room temperature before beginning the procedure. Tare a 250–500ml beaker and stir bar.
2. Wearing gloves, crack each egg and separate the egg white from the yolk using the egg separator. Wipe off the residual egg white with a gauze sheet, being careful not to break the yolk. Rupture the yolk sac with a needle and allow the contents to drip into the tared beaker. Discard the yolk sac and measure the weight of the yolk.
3. Stir the yolk(s) at room temperature and slowly add 3 volumes of Precipitation Solution A. **Assume that one gram of yolk is equal to 1ml.** For example, add 150ml of Precipitation Solution A to 50ml (50g) of yolk.
Note: Frozen yolks will be somewhat thicker after thawing and may require additional mixing.
4. Continue stirring the yolk mixture for 5 minutes to precipitate the lipids; then centrifuge the mixture at $10,000 \times g$ for 15 minutes at 4°C. (Increase time for lower centrifugal forces.)
5. Collect the supernatant into a graduated cylinder by filtering it through 4 layers (1 folded sheet) of gauze. Squeeze the gauze sheet to enhance recovery. The supernatant should be a colorless, translucent fluid. Discard the pellet. Measure the volume of the supernatant and then transfer it to a clean beaker containing a clean stir bar.

Note: If particulates are present, repeat the centrifugation and filtration (Steps 3, 4 and 5).

3.A. IgY Purification Protocol (continued)

6. Stir the supernatant at room temperature and slowly add 1/3 volume of Precipitation Solution B. For example, add 50ml of Precipitation Solution B to 150ml of supernatant.
7. Continue stirring the mixture for 5 minutes to precipitate the IgY; then centrifuge the mixture at $10,000 \times g$ for 15 minutes at 4°C. (Increase time for lower centrifugal forces.)
8. Pour off the supernatant and discard. Resuspend the IgY pellet in a volume of PBS or IgY storage buffer equal to the **original volume** of the egg yolk. For example, if the initial yolk volume was 50ml, resuspend in 50ml.

Note: IgY can be reconstituted in buffers other than PBS or IgY storage buffer.

To resuspend the pellet, gently vortex or mix with a rubber policeman. Alternatively, resuspend the pellet by capping the tube and rocking at room temperature for up to 4 hours. The IgY concentration will be approximately 2–5mg/ml and 75% pure.

9. **Optional:** Repeat Steps 6–8 to increase IgY purity to approximately 90% after resuspension (see Figure 1).
10. Store working solutions of purified IgY in PBS or IgY storage buffer at 4°C, where they are typically stable for 6–12 months without loss of biological activity. We recommend filter-sterilization for storage longer than 2 weeks.

For long-term storage, dilute purified IgY to 2mg/ml in IgY storage buffer or 0.5mg/ml in PBS and store frozen at –20°C or –70°C. IgY solutions at higher concentrations may precipitate when frozen. After thawing frozen IgY, add gentamicin sulfate to 50µg/ml to prevent bacterial contamination.

3.B. Analysis of IgY Concentration and Purity

The protein concentration of the purified IgY may be determined with a Bradford protein assay. Use Chicken IgY, Control Immunoglobulin (Cat.# G1161), to generate the standard curve. Alternatively, concentration may be determined from the absorbance at 280nm, using 1.33 as the extinction coefficient (i.e., $A_{280}/1.33 = \text{concentration in mg/ml}$) (7).

To assess the purity of the IgY preparation, analyze a 2µg sample by non-reducing 8%, 10% or gradient SDS-PAGE. A nonreducing gel is recommended because the major potential contaminants are approximately 30 and 70kDa. On a reducing gel, the heavy and light chains of IgY migrate close to these bands.

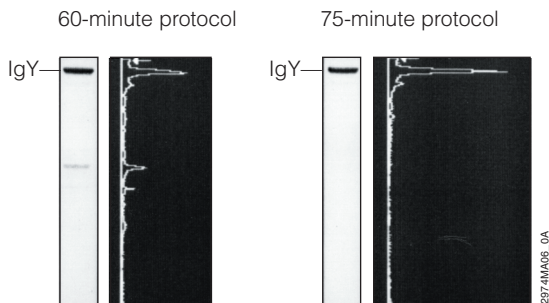


Figure 1. Chicken IgY purified with the EGGstrac[®] IgY Purification System. Two micrograms of total protein were run on a 4–20% nonreducing SDS-PAGE gel and stained with a Coomassie[®] blue staining solution. **Left:** IgY was purified following the 60-minute protocol. **Right:** IgY was purified following the 75-minute protocol, which includes the optional second precipitation with Precipitation Solution B. Densitometry tracings of the gel were generated using an AMBIS[™] Optical Imaging System.

4. Composition of Buffers and Solutions

PBS (1L)

0.2g	3mM KCl
8.0g	137mM NaCl
0.2g	1.5mM KH ₂ PO ₄
1.15g	8mM Na ₂ HPO ₄

Dissolve components in 800ml deionized water and bring to 1L. The pH should be approximately 7.4.

IgY storage buffer (1L)

1.7g	0.01M K ₂ HPO ₄ (pH 7.4)
5.8g	0.1M NaCl
50mg	50µg/ml gentamicin sulfate

Dissolve the components in 1L of deionized water. The pH should be approximately 7.4. Store at 4°C. If this buffer is frozen, add gentamicin after thawing, as freeze-thaw cycles decrease gentamicin efficacy.

5. Related Products

Product	Size	Cat.#
Anti-Chicken IgY, AP Conjugate*	100µl	G1151
Anti-Chicken IgY, HRP Conjugate	300µl	G1351
Anti-Chicken IgY, FITC Conjugate*	0.5mg	G2691
Anti-Chicken IgY, Biotin Conjugate*	0.5mg	G2891
Chicken IgY, Control Immunoglobulin*	1mg	G1161

*For Laboratory Use.

6. References

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4. Akita, E.M. and Nakai, S. (1992) Immunoglobins from egg yolk: Isolation and purification. *J. Food Sci.* **57**, 629.
5. Akita, E.M. and Nakai, S. (1993) Comparison of four purification methods for the production of immunoglobins from eggs laid by hens immunized with an enterotoxigenic *E. coli* strain. *J. Immunol. Methods* **160**, 207-14.
6. Danielpour, D. (1993) Improved sandwich enzyme-linked immunosorbent assays for transforming growth factor beta 1. *J. Immunol. Methods* **158**, 17-25.
7. Leslie, G.A. and Clem, L.W. (1969) Phylogeny of immunoglobulin structure and function. 3. Immunoglobins of the chicken. *J. Exp. Med.* **130**, 1337-52.

7. EGGStract® IgY Purification System Batch Record

Operator name: _____ Date: _____

Precipitation Solution A lot #: _____

Precipitation Solution B lot #: _____

Antigen/Project:

1. Number of eggs used: _____
2. Tare weight of beaker + stir bar: _____
3. Weight of yolk recovered: _____ (total weight - tare weight of beaker)
(1g of yolk = 1ml of yolk)
4. Volume of yolk recovered _____ ml (1g yolk = 1ml yolk)
5. Volume of Precipitation Solution A used (3 × volume in Step 3, above): _____
6. Stir at room temperature for 5 minutes. Start time: _____
End time: _____
7. Centrifuge 4°C, 10,000 × g for 15 minutes. Centrifuge & Rotor: _____
Speed: _____
Start time: _____
End time: _____

-
8. Volume of supernatant recovered: _____
9. Volume of Precipitation Solution B used ($1/3 \times$ volume in Step 7, above): _____
10. Stir at room temperature for 5 minutes. Start time: _____
End time: _____
11. Centrifuge 4°C , $10,000 \times g$ for 15 minutes. Centrifuge & Rotor: _____
Speed: _____
Start time: _____
End time: _____
12. Volume of buffer to reconstitute IgY pellet: _____
(equal to volume in Step 3, above)

Optional precipitation step:

13. Volume of Precipitation Solution B used ($1/3 \times$ volume in Step 3, above): _____
14. Stir at room temperature for 5 minutes. Start time: _____
End time: _____
15. Centrifuge 4°C , $10,000 \times g$ for 15 minutes. Centrifuge & Rotor: _____
Speed: _____
Start time: _____
End time: _____
16. Volume of buffer to reconstitute IgY pellet: _____
(equal to volume in Step 3, above)

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