

Why IgY? Chicken Polyclonal Antibody, An Appealing Alternative

Mary Haak-Frendscho
Promega Corporation

The eggs of immunized chickens are an economical and abundant source of polyclonal antibody. Chicken IgY antibody can be purified from egg yolks using the EGGstract™ IgY Purification System. This easy, rapid and efficient system results in 75% pure IgY in one hour, 90% pure IgY in 75 minutes. Anti-IgY-Alkaline Phosphatase Conjugate, for use in Westerns and ELISAs; Anti-IgY, Immobilized, for use in immunoprecipitations; and IgY control protein are also available for investigators using IgY in their research.

Introduction

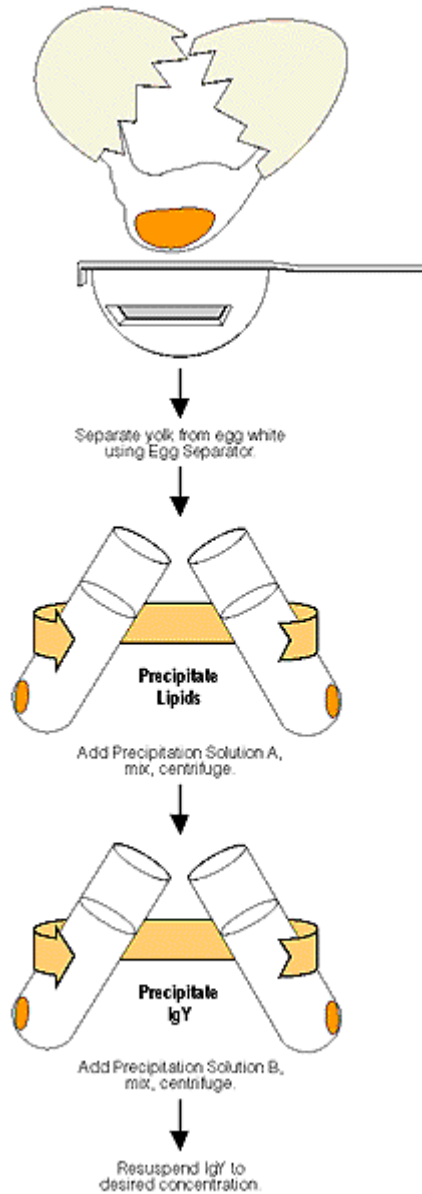
The yolk of eggs laid by immunized chickens has been recognized as an excellent source of polyclonal antibodies (pAb) for over a decade (1, 2). Specific antibodies produced in chickens offer several important advantages over producing antibodies in other mammals. Because a single egg contains as much antibody as an average bleed from a rabbit, this simple, non-invasive approach presents an appealing alternative to conventional pAb production methods. Purification of chicken egg yolk immunoglobulin Y (IgY), the 150kDa IgG homolog, does not require animal bleeding. In addition, the eggs from immunized chickens provide a continual, daily source of pAb, and this convenient approach offers greater compatibility with animal protection regulations (3). Due to the phylogenetic distance between birds and mammals, there is greater potential of producing a higher percentage of specific antibody against mammalian antigens when using chickens (2, 3). Highly conserved mammalian proteins sometimes fail to illicit a humoral immune response in animals, such as rabbits, that are traditionally used for generating pAb (4, 5). Since chicken IgY does not cross-react with mammalian IgG (6) and does not bind bacterial or mammalian Fc receptors (7), non-specific binding is reduced, and the need for cross-species immunoabsorptions is also eliminated (8).

Immunization strategy

Immunization of chickens for pAb production is comparable to that of rabbits with respect to route of injection, the amount of antigen used and the kinetics of specific antibody generation (9). Laying hens (we recommend white Leghorns because of their large eggs) are injected subcutaneously at multiple sites in the breast with 20-500µg antigen in complete Freund's adjuvant. Specific antibody titers are boosted by two or three additional injections at two week intervals. Eggs (usually one egg is laid per day) are collected daily and marked for identification. They can be stored for up to one year at 4°C prior to IgY purification. Typically, each egg will contain about 90-100mg of total IgY, and the specific antibody generally comprises 1-10% of that total, or about 1-10mg of specific IgY per egg.

Purification of IgY

The large amount of lipids present in egg yolk (composed essentially of lipoprotein and phospholipid granules [10] dispersed in soluble lipoproteins and low density lipoproteins [11]) and the failure of IgY to bind staphylococcal protein A and streptococcal protein G (7) have presented problems in the purification of chicken IgY. Several methods have been described for the purification of IgY from chicken egg yolks (1-3, 12-14), however, these protocols consist of numerous time-consuming steps, including salting in, chromatography and dialysis. In contrast, Promega's EGGstract IgY Purification System provides a rapid and easy method for the isolation of IgY, overcoming many of these problems (Figure 1). Using this system, total IgY yields following a single precipitation typically range between 55-80mg of 75% pure IgY per egg, depending on batch size, (Figure 2). A second precipitation, which requires an additional 15 minutes, will increase purity to about 90% without substantial loss on recovery of IgY (Figure 2). The yield of IgY is improved when more than one egg is processed.



1.

Figure 1. Simplified diagram of the EGGstract purification procedure.

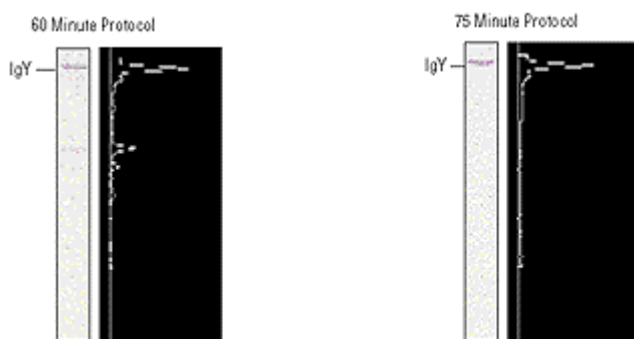


Figure 2. Chicken IgY purified with the EGGstract IgY Purification System. Two micrograms of total protein were run on a 4-20% SDS-PAGE non-reducing gel and stained with Fast Coomassie® Stain (Cat.# V5301). Left: IgY was purified following the 60 minute protocol. Right: IgY was purified following the 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.

IgY purified by this method is suitable for either affinity purification, if desired, or can be used directly for immunoblotting, ELISA and other standard immunological techniques. Binding of IgY can be detected in enzyme immunoassays with Promega's high quality, affinity-purified Alkaline Phosphatase Conjugated Goat Anti-IgY. Chicken IgY can also be used for immunoprecipitation using Promega's Anti-Chicken IgY, Immobilized in place of protein A or protein G.

Summary

Chicken IgY has considerable advantages over pAb from mammals. Promega's new EGGstract IgY Purification System provides a rapid and easy method for the isolation of IgY. The protocol and reagents supplied in the EGGstract System allow the user to recover 55-80mg of 75% pure IgY per egg yolk in about one hour. If desired, a second round of precipitation will increase the purity to about 90%. IgY purified with the EGGstract System can be used in standard immunological assays in conjunction with Promega's affinity purified Alkaline Phosphatase Conjugated Goat Anti-Chicken IgY and with Promega's Anti-Chicken IgY, Immobilized.

References

1. Polson, A.T., Von Wechmar, M.B. and Van Regenmortel, M.H.V. (1980) *Immunol. Commun.* **9**, 475.
2. Jensenius, J.C. *et al.* (1981) *J. Immunol. Meth.* **46**, 63.
3. Akita, E.M. and Nakai, S. (1992) *J. Food Sci.* **57**, 629.
4. Goueli, S.A. *et al.* (1990) *Biochem. Internat.* **21**, 685.
5. Danielpour, D. (1993) *J. Immunol. Meth.* **158**, 17.
6. Ambrosius, H. and Hadge, D. (1987) *Vet. Immunol. Immunopathol.* **17**, 57.
7. Larsson, A. and Sjoquist, J. (1988) *J. Immunol. Meth.* **108**, 205.
8. Larsson, A. and Sjoquist, J. (1990) *Comp. Immun. Microbiol. Infect. Dis.* **13**, 199.
9. Song, C.S. *et al.* (1985) *J. Immunol.* **135**, 3354.
10. Burley, R.W. and Cook, W.H. (1961) *Can. J. Biochem. Physiol.* **39**, 1295.
11. McCully, K.A., Mok, C.C. and Common, R.H. (1962) *Can. J. Biochem. Physiol.* **40**, 937.
12. Akita, E.M. and Nakai, S. (1993) *J. Immunol. Meth.* **160**, 207.

13. Hassl, A. and Aspöck, H. (1988) *J. Immunol. Meth.* **110**, 225.
14. Hatta, H.O., Sim, J.S. and Nakai, S. (1988) *J. Food Sci.* **53**, 425.

Ordering Information

Product	Size	Cat.#
EGGstract™ IgY Purification System	6 preps	G2610
Anti-Chicken IgY, AP Conjugate	100µl	G1151
Anti-Chicken IgY, Immobilized	1ml	G1191
Chicken IgY, Control Immunoglobulin	1mg	G1161

©1994 Promega Corporation. All Rights Reserved.