

(RESEARCH ARTICLE)



## Isolation of IgG from chicken serum using a combination of coconut caprylic triglyceride and ammonium sulfate precipitation

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### Abstract

This study aimed to isolate IgG from chicken serum using a combination of coconut caprylic triglyceride and ammonium sulfate precipitation techniques. This study used serum from chickens that had previously been vaccinated repeatedly using a commercial rabies vaccine. Initially, the serum was treated with 2.5% coconut caprylic triglyceride, followed by serum IgG precipitation using 40% ammonium sulfate. The 10% SDS-PAGE analysis showed that a protein of about 190 kDa with a purity above 95% was successfully isolated. Whether the isolate protein is IgG and specific to rabies antigen still needs to be investigated further.

**Keywords:** Antibody; Coconut caprylic triglyceride; Chicken serum; IgG; IgY

### 1. Introduction

The development of technology that utilizes the uniqueness of antibodies has recently developed rapidly, both for immunodiagnosics and immunotherapy [1, 2]. The antibodies generally used in immunodiagnosics are polyclonal antibodies produced from mammals and birds, monoclonal antibodies, antibodies resulting from engineered technology, or a combination of them [3-5]. Antibodies from poultry are known as yolk immunoglobulin (IgY), while the most common isotype of immunoglobulin in mammals is IgG [6].

There are several advantages of IgY compared to IgG from mammals. One of the most prominent is that IgY does not interact with mammalian Fc receptors, rheumatoid factor, and components of the complement system; hence the risk of further inflammation is minimal. The uniqueness of IgY is due to the evolutionary distance between mammals and chickens [7], [8]. Such uniqueness has made avian immunoglobulins attractive to researchers, both for diagnostic and immunotherapeutic development [9].

From an animal welfare aspect, harvesting yolk is more animalistic than harvesting blood from mammals because it is less invasive. That is why, recently, the utilization of laying hens as immunoglobulin producers has received wider attention. Methods to isolate IgY from chicken eggs have recently been intensified [10]. On the other hand, methods to isolate immunoglobulin from chicken serum have not been widely explored. This is understandable because, practically, the antibodies obtained are less than those from yolk, which can reach around 40 g/hen/year [11, 12].

The idea of utilizing immunoglobulins from chicken serum, which can be obtained in abundance when IgY production is optimal from a laying flock, especially those that hens will be culled i.e. through slaughter procedures, deserves attention. Therefore, a practical method of isolating and purifying IgG from the serum of laying hens is needed. Here we report an attempt to isolate IgG from chicken serum through a fractionation process using a combination of coconut caprylic triglyceride and ammonium sulfate. This method is quite classical but is still being explored for isolating IgG from mammalian serum [13, 14], as well as from laying hens [15]. Caprylic acid is more commonly used to extract IgY

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from egg yolk, as recently reported by Redwan et al. [9], rather than from poultry serum. However, the use of caprylic acid needs to be investigated continuously, especially during the culling period, so that the obtained hens' serum can be optimally utilized as a potential IgG source.

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## 2. Materials and methods

### 2.1. Chicken Serum

In this study, the serum used was from laying hens that had been repeatedly vaccinated using a commonly used rabies vaccine (Defensor3). Vaccination was conducted three times with a three-week interval between each vaccination. Chicken serum was collected two weeks after the last vaccination. The study was conducted following animal welfare regulations at the Faculty of Animal Science, University of Mataram, Indonesia.

### 2.2. Isolation of Chicken IgG

The method used to isolate IgG from chicken serum was according to [9, 15] with slight modifications. Two milliliters of chicken serum were diluted using 4x the volume (8 ml 0.1 M citric acid, pH 4.0), and the pH was adjusted to 4.5 with a 1.5 M Tris base. Subsequently, coconut caprylic triglyceride (locally produced, "Happy Green" Indonesia) was added gradually to reach a final concentration of 2.5% in diluted serum. The mixture was vortexed using "TOMY, microtube mixer MT-360" at two mixing speeds for 30 minutes at room temperature; then the solution was centrifuged at 10000 rpm, 4 °C for 30 minutes (TOMY MDX high-speed refrigerated microcentrifuge). The supernatant was collected and filtered using a 0.22 µm filter, and then the pH was adjusted to 7.4 with a 1.5 M Tris base.

The next step was precipitation using ammonium sulfate by gradually adding saturated ammonium sulfate pH 7.4 to the filtered supernatant of the chicken serum-coconut caprylic triglyceride mixture at 0.667 ml per 1 ml of the filtered solution. The mixture was shaken for 30 min on ice and then centrifuged at 10000 rpm, 4 °C for 30 min. The precipitate formed was washed using 2 ml of 40% ammonium sulfate and centrifuged at 10000 rpm, 4° C for 30 min. Finally, the pellet was resuspended in 0.5 ml of 1x PBS and dialyzed for 18 hours at 4 °C against PBS pH 7.4 with three changes of buffer.

### 2.3. SDS-PAGE analysis

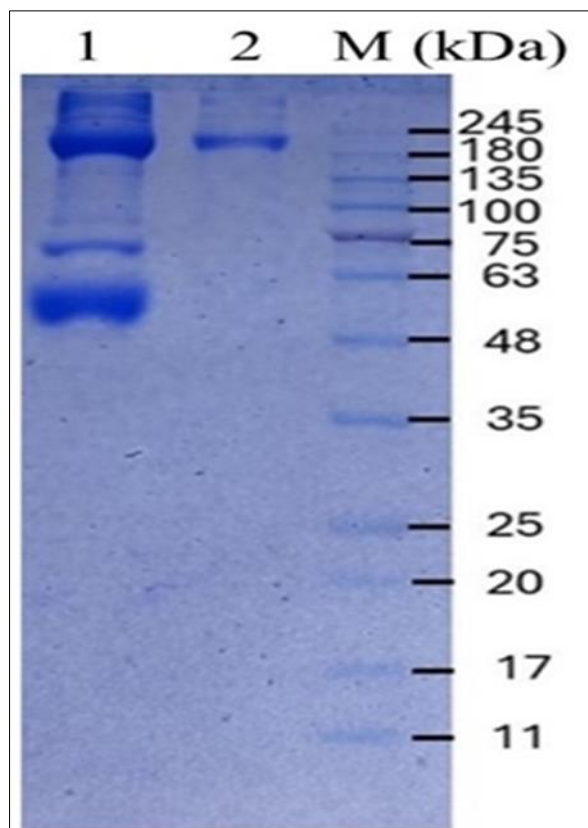
SDS-PAGE 10% was applied to analyze the purity level and estimate the isolated protein's molecular weight. SDS-PAGE was prepared based on Laemli [16], modified by Kisworo and Depamede [17] and Nurhaerani et al. [18].

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## 3. Results and Discussions

This study aimed to isolate IgG from the serum of chickens previously vaccinated repeatedly using a commercial rabies vaccine (Defensor3). The utilization of immunoglobulin from chicken serum is limited because the harvesting process is technically impractical. However, as a by-product of the IgY production process from chicken eggs, the volume of serum that can be collected during the culling period is significant. Therefore, efforts to isolate immunoglobulins from chickens that produce IgY according to the targeted antibodies are worth considering.

Several procedures to isolate immunoglobulins from serum have been studied, especially those from mammalian serum. For immunoglobulins from chicken, generally derived from yolk (IgY), among others, the isolation method is a combination of caprylic acid and ammonium sulfate [15]. The combination method is also used for the extraction of IgG from mammalian serum [13] [14]. In this study, we successfully isolated immunoglobulins from the serum of chickens vaccinated with a commercial rabies vaccine (Defensor3), as shown in Figure 1.



**Figure 1** SDS-PAGE (10%) of the unpurified anti-rabies chicken serum (lane 1) and isolated IgG anti-rabies antibody of chicken serum (lane 2) obtained in this study. Lane M contains a protein molecular weight marker (GangNam stain, Intron\_Biotech, Inc.)

It is clear in Figure 1 that based on a 10% SDS-PAGE analysis, a protein from chicken serum with a purity close to 100% (lane 2) relative to the unpurified serum (lane 1) was obtained in this study. Based on the relative molecular weight markers, the purified protein is about 190 kDa. At lane 1, there are two strongly expressed serum protein bands, i.e. 190 kDa and a molecular weight of about 60 kDa. Following the treatment with coconut caprylic triglyceride and 40% ammonium sulfate, proteins with a molecular weight of 60 kDa were eluted.

Amro et al. [19] reported two molecular weights of IgY from chicken egg yolk purified using PEG 6000. The first was 65 kDa for the heavy chain and 27 kDa for the light chain. In our study, no protein with a size of 27 kDa was observed; instead, it was 190 kDa and 60 kDa. The 60 kDa protein is the closest molecular weight to the IgY heavy chain of 65 kDa reported by Amro et al. [19]. However, in this study, the proteins were eluted.

Ruhi et al. [20] reported the molecular weight of IgY from egg yolk was 181.55 kDa, while anti-rabies IgY isolated using a combination of NaCl, PEG6000, and 40% ammonium sulfate produced an IgY protein weights of 164.16 kDa. These sizes are lower than our results but more than twice the molecular weights of the IgY heavy chain reported by Amro [19]. This is probably because we isolated immunoglobulins from chicken serum instead of yolk origin. One of the classic references related to the size of the IgG molecular weight from chicken serum should be considered as a comparison, i.e., which weighs up to 206 kDa [21], that close to our results of 190 kDa. To ensure that the protein we isolated is chicken serum IgG that is specific against the Defensor3 rabies vaccine, the immunoassay is currently being carried out.

#### 4. Conclusion

Immunoglobulin (IgG) with a size of 190 kDa was successfully isolated from chicken serum using a combination of local coconut caprylic triglyceride and 40% ammonium sulfate. The immuno-specificity of the isolated IgG still needs to be studied further.

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## Compliance with ethical standards

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### Disclosure of conflict of interest

No conflict of interest is to be disclosed.

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