Chicken Eggs IgY purification Work Sheet (make more copies if nec

Date kits used: _____: **Technician**: _____

Project Name: _____

Date Eggs Collected: _____: **Total # used for IgY**: ____

Beaker/Petri dish/boat Tare wt: _____

Weight with the yolks: _____gms: **Egg yolk volume**: _____

Water added: _____mls; **IgY pption buffer added** _____

1st Delipidatioin/pption time: From _____ To: _____

IgY Supernatant or filtrate collected vol (step B.iii) : _____

2nd IgY pption buffer added (step C.i): _____

2nd IgY precipitation time/incubation at 4oC: From _____ To: __

IgY Centrifugation time: From _____ to: _____

Volume of IgY reconstitution buffer added: _____-_____

IgY dilution for A280: _____ul in _____ uls; Dilution factor:____

A280: _____; **IgY Concn**: _____ (A_{280} 1.400=1 m

# of aliquots made and storage: _____

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ADI also provides many related reagents and kits for chicken IgY

20010-1	Control Chicken (non-immune) **serum IgG**, purified
6010	Chicken Egg **Ovalbumin** ELISA kit, 96 tests
6020	Chicken **Serum IgG** (total) **ELISA kit**, 96 tests
6040	Chicken **Serum IgM ELISA kit**, 96 tests
6030	Chicken **IgY** (**egg yolk**) **ELISA kit**, 96 tests
60320	Goat Anti-Chicken IgG (H+L)-HRP conjugate
60326-AM	Goat Anti-Chicken IgG (H+L)-Agarose
60360	Goat Anti-Chicken IgG (H+L)-FITC conjugate
80176	ELISA Kit for the detection of Chicken Antibodies
80220	Horseradish peroxidase (HRP) conjugation kit for ant
80300	Protein (antigen/antibodies) Biotinylation Kit

Instruction Manual No. M-60100

YolkY ™ Chicken Egg Yolk IgY antibody purification kit

Cat. No. 60100

For IgY antibody purification from Chicken Eggs
(also applicable for the purification of IgY from
Turkey, Quail and other Egg Yolks)

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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LIFE TECH **Life Technologies**™
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YolkY ™ Chicken Egg Yolk IgY Purification Kit

Cat. No. 60100

Kit Contents: (reagents for IgY purification from 6-8 eggs)

Components	Cat. #
YolkY ® Egg yolk separator, 1 (re-usable) 	60101
IgY Precipitation buffer concentrate, 125 mls	60102
Filters, 12	60103
IgY Reconstitution Buffer concn (20 X), 10 ml	60104
Complete Instruction Manual	M - 6 0 1 0 0

The use of chicken antibodies (egg yolks) in biomedical research is increasing due to many advantages associated with chicken IgY. Unlike antiserum, egg yolk cannot be directly used for analyses. Egg yolk contains large amount of lipids (lipoproteins and phosvitin granules mixed with livetins and low-density lipoproteins) making their direct use in immunoassays problematic. ADI's **YolkY ™ Egg yolk IgY purification kit** is designed to purify IgY from egg yolk in about 30 min hands-on time and just 1 or 2 centrifugation. Using the YolkY ™ Chicken Egg Yolk IgY Purification Kit , total IgY yields range between 70-100 mg (6-8 mg/ml of the yolk) of ~90% pure IgY per egg. Chicken IgY purified by this kit is suitable for direct use in ELISA, Western or other immunological assays. Specific IgY antibody (against the immunized antigen) will be approx. 1-10% of the total IgY and it can be easily purified from the total IgY by affinity chromatography using the antigen coupled to agarose.

Add the IgY precipitation buffer slowly to the egg yolk supernatant. Gently mix the solution for 15-30 min at room temp. If this step is being performed in a tube then the solution can be mixed manually. It is necessary to leave the mixed solution for at least 2 hours (several hours to overnight is preferred) to complete the IgY precipitation step. Slightly turbid white solution will be obtained.

ii) Centrifuge the solution at 15, 000 rpm for 30 min at 4oC. Carefully decant or remove the clear supernatant. Invert the centrifuge tube to completely remove the liquid. Discard the clear supernatant and keep the precipitate (IgY).

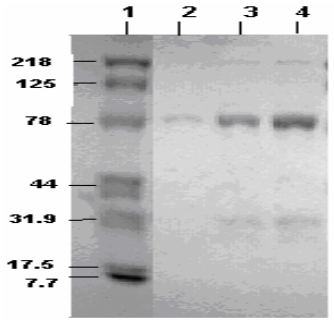
Notes: If the supernatant is not clear then it can be centrifuged the 2nd time at higher speed or for longer time (60 min).

iii) Dilute IgY reconstitution buffer concentrate 1:20 with water (1 ml of conc and 19 ml water) to prepare 1X solution. Dissolve the precipitate in 1 X IgY reconstitution buffer in the original volume of the egg yolk (if the starting egg yolk volume was 25 mls from 2 yolks then use 25 mls of IgY buffer). More buffer can be used to dissolve IgY if solution remains turbid.

iv) Determine purified total IgY concentration by measuring Absorbance at 280 nm using water as blank (A280 of 1.400 =1 mg). Typically 1:10 (100 ul in 1 ml water) or 1:20 (50 ul in 1 ml) is necessary for Absorbance. Multiply the calculated concn with the dilution factor. An average non-immunized eggs contain approx. 6-8 mg/ml IgY.

v) The purified IgY is typically ~90% pure. This purity is more than sufficient for most immunological assays or for affinity purification. If necessary, the IgY can be re-precipitate as in steps C.i-C.iv.

vi) Appropriate anti-bacteriostatic agents (0.1% azide or 0.02% sodium merthiolate) can be added. Azide is an inhibitor of HRP. Do not add azide if the antibody needs to be coupled to HRP or incubated together with the 2-ab-HRP conjugates. Store antibody in suitable size aliquots (1-5 ml) at –20oC or below. Purified IgY is stable for years.



SDS-PAGE of purified chicken IgY under reducing conditions. Lane 1, Mol Wt marker; Lanes 2 (1 ug), Lane 3 (5 ug), and lane 5 (10 ug) of purified IgY. Two major bands of Heavy and light chains are visible. Purity has been assessed ~90%.

i) Add required volume of water to the egg yolks and mix in the tube by vortexing the solution for 15-30 seconds at room temp. It is also possible to use a beaker and mix the yolk and water using a stir bars. You will get a homogenous solution without the presence of large clumps of yolks.

Notes: We assumed an average yolk volume of 10-15 mls per egg. Approx. 3 times of water is being added (30-35 mls) to bring it to a total volume of 45 mls. If there is less yolk or more yolk (eg. Turkey eggs are twice as big) then water volume can be adjusted accordingly to make at 3-4 times the initial egg yolk volume.

ii) Slowly add required volume of IgY precipitation buffer (e.g., 5 ml per yolk) and mix it for 15-30 min. A significant amount of the precipitate or large clumps will form. It is important to leave the solution for at least 1 h at 4oC to completion of the de-lipidation process. We recommend the solution to be left overnight at 4oC if there is no hurry to get the process done right away.

iii) Wet the filters (2 sheets) with water. Filter the solution and collect the clear filtrate (~35 mls per 50 mls). Discard the filters. Usually the filtrate is quite clear and it can be used for the next step. If there is significant precipitate then the solution can be filtered again using a fresh sheets of filters. Clear supernatant can be left at 4oC overnight if necessary.

Notes: If filtration does not make the solution clear then it can be centrifuged at 4oC (10, 000 rpm, 20-30 min). Collect the clear supernatant.

C. Precipitation of IgY

i) Precipitate IgY with the IgY precipitation buffer concn using 1/3 the volume of supernatant obtained in step B.III. Following table can be used to determine the amount of the IgY precipitation buffer.

Volume of Yolk Supt. (Step B.III)	IgY Precipitation Buffer Concn.
30 mls	10 ml
35 mls	12 ml
70 mls	24 mls
100 mls	34 mls

Notes: An average of 35 mls supt will be obtained from 1 average egg yolk requiring 12 mls of the IgY precipitation buffer. Adjust the volume of the IgY precipitation buffer as needed.

Introduction

IgG's are produced by mammals. Lower vertebrates (reptiles, amphibian, and birds) produce an equivalent of mammalian IgG known as IgY (from egg Yolk). The IgY has the same general structure as mammalian IgG with 2 heavy chains known as "nu" (~65-70 kDa) and 2 light chains (22-30 kDa). IgY, mol wt ~180 kDa, runs as a broad band because of the presence of substantial amount of carbohydrates (approx. 3%). The nu chains have 4 constant and 1 variable domains. However, IgY lacks the "Fc" domain and can not fix complement or bind to protein-A or G. So Protein A/G can not be directly used to purify IgY or used in immunoprecipitation. Therefore, it will be necessary to modify the IP assay and use another mammalian derived (rabbit/goat etc) anti-IgY and then use Protein A/G for IP's. Nonetheless, using appropriate secondary antibodies (anti-chicken IgY-Peroxidase or Phosphatase or FITC conjugates), chicken antibodies can be used in most immunological assays.

Since chicken antibodies do not crossreact with mammalian IgGs, bind to Fc receptors, interact with rheumatoid factors or react with HAMA (human anti-murine antibodies), non-specific binding of chicken antibodies is greatly reduced in most immunological assays. In addition, unlike most mammalian secondary antibody-enzyme conjugates, it is not necessary to adsorb the chicken antibody-enzyme conjugate with mammalian protein to reduce background.

Chickens are immunized using the same general method as used when utilizing rabbits or goats. Injections are given at multiple sites in the breast muscle. It takes at least 2 weeks or more to initiate immune response. Antibodies are produced and released into serum. Antibody titer increases during subsequent immunization. Although, one may collect chicken serum, it is preferred not to stress the birds since laying hens also concentrate antibodies in the egg yolk. Laying hens produce eggs almost on a daily basis and harvesting eggs is a very noninvasive method of collecting antibodies. On an average, a single egg may contain the equivalent of a standard rabbit bleed, harvesting eggs greatly enhances the amount of antibodies that can be collected from a single chicken.

Typically rabbits are bled every 2 weeks yielding ~15-20 ml serum whereas chicken may lay 12-15 eggs during the same 2-week period. Therefore, antibody production in chicken can be 15-20 times of that obtained in rabbit. Antibody concentration in the egg yolk is higher than what is found in chicken serum. This high concentration of antibodies is presumably used to confer passive immunity to newly hatched chicks. It is estimated that a single egg may contain ~70-100 mg total antibodies of which (1-10%) may represent the specific antibodies.

Purification of chicken IgY from egg yolk and storage of antibodies

Egg yolk contains large amount of lipids (lipoproteins and phosvitin granules mixed with livetins and low-density lipoproteins) making their direct use in immunoassays problematic. Yolky egg yolk IgY purification kit is designed to

purify IgY from egg yolk in about 30 min hands-on time and just 1 centrifugation. There are enough reagents to purify IgY from at least 10-12 standard size chicken eggs. Purified IgY is typically 80-90% pure and it is suitable for most immunological applications (ELISA, Western, IHC etc).

Partially purified IgY is quite stable at 4oC for years provided a bacteriostatic agent (Azide or Merthiolate) has been added to prevent microbial growth. The core body temperature of chicken is about 41oC and half-lives of chicken antibodies is in months. Therefore chicken antibodies are also quite stable at room temperature for months. It is, however, advisable to freeze antibodies in suitable aliquots and avoid freeze and thaw. Freeze and thaw will do more harms to antibodies than leaving them at 4oC for weeks and months.

MATERIALS AND EQUIPMENT REQUIRED (not supplied)

Test tubes (15, 50 mls), Beakers (100-500 mls), conical Funnel, Petri dish, Pipettes 5, 10 ml, Adjustable micropipet (25-100 ul), Centrifuge (15, 000 rpm), Spectrophotometer; stir bar and stir plates

LIMITATIONS

The Alpha Diagnostic Intl. IgY purification kit is for in vitro research use only.

STORAGE AND STABILITY

All reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually six months from the date of shipping, under appropriate storage conditions.

Detailed IgY Egg yolk antibody purification protocol

This process is recommended for purification of IgY from 2 chicken egg yolk. It can be scaled up accordingly if more eggs are processed.

A. Egg Collection, storage and Separation of IgY

i) Whole eggs (non-immune or immune) can stored at 4oC for up to 6-8 weeks. For longer periods, it is better to collect the egg yolks and store it frozen.

ii) You will need a weighing boat or a Petri dish or an empty clean glass beaker of appropriate size to collect egg yolk. Record the tare weight of the container used for egg yolk collection. Crack open the eggs carefully without puncturing the egg yolk. Use the **Yolky egg yolk separator** to separate the yolk white. The yolk will stay in

the cup and the white will run out or allowed to run out as much as possible. Transfer the yolks on the weigh boat or Petri dish and wash with distilled water. The yolks can be adsorbed on a paper towel to remove the adhering egg while. **Notes**: Egg yolk separator is re-usable after washing.

iii) Carefully roll the egg sac onto a clean, dry paper towel and position the egg sac near the edge of one side of the towel. Suspend the egg sac in the paper towel over the tared beaker or weigh boats or Petri dish. Puncture the egg sac with the Pasteur pipette or a 1 ml pipette tips and collect as much of the egg yolk as possible. Discard the paper towel. It is possible to collect yolk from a pool of eggs (pre-immune or immune eggs).

iv) Reweigh the beaker and record the weight (volume) of the egg yolk. A standard size chicken egg will contain approx. 10-15 mls or 10-15 g (1 ml=1 g). It is not obligatory to accurately weigh the yolk if the purpose is to simply purify IgY for use in Western or ELISA.

B. De-lipidation of IgY from Egg yolk

iv) Add sufficient volume of distilled water to bring the total volume 45 mls (example, if egg yolk volume as about 10 mls then add water, ~35 mls, to a total of 45 mls). For 2 egg yolks it will be 90 ml total. Therefore it is not necessary to know what was the egg yolk volume. Use the following table to determine the amounts of various solution.

# of eggs **(Yolk Volume)**	**Add Distilled Water up to: (including the yolk)**	**IgY Precipitation Buffer Concn**
1 (10 mls)	Total 45 mls	5 mls
2 (20 mls)	Total 90 mls	10 mls
3 (30 mls)	Total 135 mls	15 mls
4 (40 mls)	Total 180 mls	20 mls
5 (50 mls)	Total 225 mls	25 mls
6 (60 mls)	Total 270 mls	30 mls

Notes: It is easier to do the above step in a 50 ml tube (1 tube for each egg yolk) or combine the yolks and use a an appropriate size measuring cylinder. If the egg yolk volume if not known, one can still suspend it in a total volume of 45 ml in water. If the yolk+water volume is less than 45 mls then the IgY pption buffer must be adjusted accordingly.