EggsPress IgY Purification Kit Instruction Manual

Features

- Quick and efficient procedure.
- Purify without loss of activity.

Ordering Information

Catalog Number
IK 500
IK 2000
IK 4000
IK 8000

Size
5, 20, 40, 80 egg yolks

Format
Purification Kit

Compatible Species
Chicken

Company Information
Exalpha Biologicals, Inc.
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Other Kits & Reagents Available from Exalpha Biologicals

DNA Fragmentation Detection Kit
X2044K1 (30 Tests)
X2044K2 (60 Tests)

BrdU Chemiluminescent Cell Proliferation Assay Kit
X1623K1 (200 Tests)
X1623K2 (1000 Tests)
X1623K3 (5000 Tests)

BrdU Immunohistochemistry Kit
X1545K (50 Sections)

BrdU Fluorescent Immunohistochemistry Kit
X2839K (FL488 nm, 50 Sections)
X2840K (FL549 nm, 50 Sections)

BrdU Cell Proliferation Assay Kit
X1327K1 (200 Tests)
X1327K2 (1000 Tests)
X1327K3 (5000 Tests)

Antifade Mounting Medium
X2841 (7 ml)

BrdU Reagent for In Vivo Injection
X2834 (5 x 5 mg)

BrdU Unstained Control Slides
X2743 (5 slides)

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Intended Use

The Exalpha Biologicals, Inc. IgY EggsPress Purification Kit is for the purification of chicken antibodies (IgY) from eggs without loss of activity.

This kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage of Kit Components

Exalpha's IgY EggsPress Purification Kit components are shipped at ambient temperature. Upon receipt, store kit at room temperature. Prior to use, acclimate the reagents to 4-8°C.

Background

Exalpha's EggsPress Purification Kit enables researchers to quickly and efficiently purify chicken antibodies (IgY) from the eggs of their immunized hens without loss of activity. The disadvantage of using hens to make your antibody is the difficulty of purifying IgY from the lipid environment of the egg yolk. This problem is circumvented by using Exalpha's IgY antibody purification kit.

Principle of the Kit

Exalpha's EggsPress Purification Kit has two isolation steps — one to remove the lipid (Reagent A) and the second to precipitate the IgY (Reagent B). The purification will provide you with an IgY fraction that is at least 85% pure with a yield of 4 to 7 mg of IgY per ml egg yolk. The entire procedure takes about 4 hours but only 30 minutes of hands-on time.

Once purified, the IgY antibody can be treated like any other immunoglobulin. The antibody can be labeled or affinity-purified. Proceed as you would with any antibody fraction. It should be noted that IgY does not bind to protein A, protein G, or protein L. The IgY may be used as a primary antibody in an ELISA test, Western blot or immunostaining procedure.

Materials Provided

The EggsPress IgY Purification Kit is provided in a 5, 20, 40, or 80 egg yolk size.

5 egg yolk isolations (Cat. # IK 500)
1 each of Reagent A (Delipidation), 500 ml
1 each of Reagent B (IgY Precipitation Reagent), 500 ml
1 each of Egg Separator

20 egg yolk isolations (Cat. # IK 2000)
4 each of Reagent A (Delipidation), 500 ml
4 each of Reagent B (IgY Precipitation Reagent), 500 ml
1 each of Egg Separator

40 egg yolk isolations (Cat. # IK 4000)
1 each of Reagent A (Delipidation), 3.8 L
1 each of Reagent B (IgY Precipitation Reagent), 3.8 L
1 each of Egg Separator

80 egg yolk isolations (Cat. # IK 8000)
2 each of Reagent A (Delipidation), 3.8 L
2 each of Reagent B (IgY Precipitation Reagent), 3.8 L
1 each of Egg Separator

Materials Required But Not Provided

1. Egg(s) / Hen(s)
2. Antigen
3. Freund's complete adjuvant
4. Freund's incomplete adjuvant
5. Syringe & needle
6. Refrigerator
7. Distilled or deionized water
8. Pasteur pipette
9. Beakers & graduated cylinders
10. Balance
11. Centrifuge tubes
12. Centrifuge
13. Filter
14. Magnetic stir bar and stir plate
15. Phosphate Buffered Saline, pH 7.2 (PBS)
16. Sodium Azide
17. Paper towel(s)

Summary Protocol

1. Collect pre-immune eggs prior to immunization, then immunize the hen(s).
2. Acclimate the eggs, Reagent A, and Reagent B to 4-8°C prior to use.
3. Separate and rinse the cold egg yolk(s). Drain rinsed yolk(s) into a tared beaker.
4. Add 5 volumes of cold Reagent A and stir gently.
5. Incubate for 2-24 hours at 4-8°C.
6. Mix gently, then centrifuge at 4,000 x g for 20 minutes at 4-8°C. Collect supernatant. If particulates are present, repeat step 6 and filter if necessary.
7. Measure volume and transfer into a beaker.
8. Add equal volume of cold Reagent B while stirring. Stir for 2 minutes.

9. Incubate at 4-8°C for 1 hour.
10. Centrifuge at 4,000 x g for 20 minutes at 4-8°C and discard the supernatant.
11. Dissolve precipitate in a volume of PBS equal to the original yolk volume, then sterile filter.
12. Store at 4-8°C.

Precautions and Recommendations

1. Wear disposable gloves and eye protection.
2. Do not use the kit beyond the expiration date.
3. Do not mix reagents from different kits.
4. Do not mouth pipette or ingest any of the reagents.
5. Do not smoke, eat, or drink when performing the purification or in areas where samples or reagents are handled.

Detailed Protocol

Please note: This kit is designed to purify IgY from egg yolks that have been refrigerated for no longer than 2 months. If the egg yolks are older than this (or have been frozen), dilute with Reagent A six times (rather than 5) and incubate for 24 hours before centrifugation. Exalpha, however, is unable to assure the performance of the kit under these conditions. To store the eggs for longer than 2 months, mix yolks with 2 volumes of Reagent A, then freeze. Prior to purification, add 3 volumes of Reagent A to the thawed yolks.

1. Immunization

1a. Collect a couple of pre-immune eggs before immunization begins.

1b. On day 0, inject between 0.02 and 0.5 mg antigen (with Freund's complete adjuvant)

subcutaneously and/or intramuscularly into the breast tissue of the hen at multiple sites. The total volume of antigen/adjuvant should be about 1 ml with the adjuvant making up between half and two-thirds the volume. Use a comparable amount of antigen that you would use to immunize a rabbit.

1c. Repeat immunizations on day 14 and 28 using incomplete Freund's adjuvant and about half the amount of antigen. Specific antibody should be detected by day 30 in the eggs. For prolonged antibody production, hens should receive boosts every couple of months.

1d. Individual hens will respond differently to immunization with a particular antigen. Antibody titers will vary as will the time period in which the titers are stable.

2. Reagent Preparation

2a. Acclimate the egg(s), Reagent A, and Reagent B to 4-8°C.

3. Yolk Separation

3a. Separate cold egg yolk from the white using the egg separator.

3b. Rinse yolk with distilled water.

3c. Puncture yolk membrane with Pasteur pipette and allow yolk to drain into tared beaker. Discard yolk membrane.

3d. Record the weight of the yolk. Assume that 1 ml of yolk is equal to 1 gram.

4. Addition of Reagent A

4a. Add 5 volumes of cold Reagent A to the yolks very slowly with continual gentle stirring until well mixed. Avoid foaming.

5. Incubation

5a. Allow diluted yolk to stand for at least 2 hours or up to 24 hours at 4-8°C.

5b. Mix gently, then add to centrifuge tubes.

6. Supernatant Collection

6a. Centrifuge tubes at 4,000 x g for 20 minutes at 4-8°C. Use longer centrifugation time for lower speed.

6b. Collect supernatant into graduated cylinder. The supernatant should be colorless and translucent.

6c. If particulates are present, repeat the centrifugation step and if necessary, filter (see troubleshooting section for more information).

7. Supernatant Transfer

7a. Measure volume.

7b. Transfer supernatant into a beaker with a stir bar.

8. Addition of Reagent B

8a. Add an amount of cold Reagent B that is equal to the volume of the supernatant in step 7a while stirring with stir bar.

8b. Continue stirring for 2 min.

9. Incubation

9a. Allow suspension to stand for at least 1 hour up to 24 hours at 4-8°C.

10. Collection of Precipitate

10a. Centrifuge at 4,000 x g for 20 minutes at 4-8°C and discard the supernatant.

11. Resuspension

11a. Dissolve precipitate in volume of PBS equal to original egg yolk volume then sterile filter.

11b. IgY concentration should be between 4 – 7 mg/ml with purity of 85% or greater.

12. Storage

12a. Store the IgY (with added preservative sodium azide at 0.1%) in the refrigerator for a year (or longer) with no loss of activity.

Troubleshooting

Following centrifugation of yolk with Reagent A (step 6), if supernatant is yellow and viscous and/or pellet is not solid:

1. Resuspend the pellet and add 1 volume of Reagent A equal to the initial egg yolk volume, mix and place in the fridge (4-8°C) overnight.
2. Centrifuge again the next day. The pellet should be solid. The supernatant may be slightly yellow and somewhat opaque but not viscous.
3. Continue with Step 7.

Following centrifugation of yolk with Reagent A (step 6), if supernatant is colorless and translucent but particulate matter is present in the supernatant:

1. Filter the supernatant. Fast-flow filter is adequate.
2. Continue with Step 7.

Following centrifugation after addition of Reagent B (step 10), if there is no white pellet and there is a diffuse white precipitate throughout:

1. This is an indication that the supernatant following centrifugation with Reagent A had too much lipid in it.
2. This can occur with older egg yolks (older than 3 months) or with frozen yolks.
3. Start again diluting your egg yolks five times with Reagent A and incubating overnight (rather than 2 hours), as described in the Summary Protocol page 6.

References

1. Young, CA, Silversides FG, SRM Jones, 2007. Aquaculture 273:398-404.
2. Allesandro, R, Gallo A, Barranco M, Principe S, Taverna G, Duro G, Cassata G, Becchi M, Fontana S and G DeLeo. 2009. Poult Sci 88: 1773-1778.
3. Janhardhana V, Tachedjian M Crameri G, Cowled C, Wang LF and ML Baker. 2012. Dev Comp Immunol 36:610-18.
4. Liu, J., Kerr, P.J., Wright, J.D. and T. Strive 2012. Serological assays to discriminate rabbit haemorrhagic disease virus from Australian non-pathogenic rabbit calicivirus. Vet. Mic. 157: 345-354.

Ordering information

Catalog Number	Size
IK500	5 egg yolk
IK2000	20 egg yolk
IK4000	40 egg yolk
IK8000	80 egg yolk

Contact Information

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