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GenWay's IgY Purification Kit

Catalog Number – GWB-IGYPUR

PURPOSE

To purify total IgY from chicken eggs.

PRINCIPLE

For preparing an immunological preparation which comprises immunising hens against a given antigen, collecting eggs of the hens thus immunised and containing antibodies against such antigen and separating the antibodies from the yolk of the eggs. This can be achieved by separating off the lipid content of the egg yolk, rendering the antibodies in the egg yolk indispersable by mixing the egg yolk with a water-soluble linear filamentary non-charged polymer precipitant and recovering the indispersable antibodies separated from the resulting supernatant.

REAGENTS

- Reagent A
 - 5 egg kit – 1 x 500 mL
 - 20 egg kit – 4 x 500 mL
 - 50 egg kit – 5 x 1L
 - 100 egg kit – 10 x 1L
- Reagent B
 - 5 egg kit – 1 x 500 mL
 - 20 egg kit – 4 x 500 mL
 - 50 egg kit – 5 x 1L
 - 100 egg kit – 10 x 1L
- Reagent C
 - 5 egg kit – 1 x 50 mL
 - 20 egg kit – 1 x 200 mL
 - 50 egg kit – 1 x 500mL
 - 100 egg kit – 1 x 1L
- Egg Separator



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PROCEDURE

1. Separate the egg yolk from the white using the egg separator. Remove as much egg white as possible using tweezers and vacuum. Transfer the clean egg yolk from the egg yolk separator into 250 ml centrifuge bottles. Each bottle can be used to treat 1 to 4 eggs.

Note: try to continue with subsequent steps no later than 15 minutes after the eggs are cracked. Otherwise, the IgY purification suffers adverse effects.

2. Mix the egg yolks thoroughly before adding Reagent A.
3. For 1 part of well-mixed egg yolk (~15 ml/egg), add 2 parts of Reagent A (30 ml), mix well.
4. Balance the bottles with Reagent A.
5. Incubate at RT for 20 minutes with shaking.
6. Centrifuge at RT for 15 minutes under 20,000 x g.
Note: Try to decant the supernatant no later than 10 minutes after the centrifugation finishes. Otherwise, the IgY purification suffers adverse effects.
7. Collect supernatant. The supernatant can be stored at 4° C overnight.
8. For 1 part of egg yolk filtrate (30 ml/egg), add 50 ml Reagent B Stock Solution. If necessary, 30 ml additional Reagent A can be added to even egg numbers for later centrifugation along with a matching additional 50 ml Reagent B.
9. Balance the bottles with Reagent A.
10. Incubate at RT for 20 minutes with shaking.
11. Centrifuge according to the following table at RT.

Egg #	Time at 14,000 g (min.)	Time at 6,800 g (min.)	Time at 5,000 g (min.)
1-2	13		
≤ 7		30	40
8-9		35	50
10-12		45	60

12. Discard the supernatant and dissolve the IgY precipitation in 10 ml per egg of Reagent C (1XPBS). The IgY concentration should be 8 -12 mg/ml. Once PBS is added to the IgY, it can be stored at 4° C overnight.