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A Novel, Rapid Procedure for Purification of IgY from Egg Yolk

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Introduction

There are several advantages in producing antibodies from avian sources (1). The immunization of chickens induces the production of specific antibodies in both serum and egg yolk in similar concentrations. It is easier to collect and store eggs compared to blood, and a few eggs per week provide the same amount of antibodies as repeated bleeding of an immunized rabbit. Because of evolutionary differences, avian species produce an elevated antibody response to highly conserved, weakly immunogenic mammalian antigens.

Here, we present a novel procedure for the purification of IgY from egg yolk based on thiophilic interaction chromatography using HiTrap™ IgY Purification HP.

Conclusions

- A rapid procedure for purification of IgY from egg yolk has been established, using HiTrap IgY Purification HP.
- The purity of IgY after one-step chromatography was judged to be 70% by SDS-PAGE and gel filtration.

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Sample preparation

Egg yolks were separated from egg whites and collected. Each egg yolk had a volume of about 20 ml. Two extraction methods were used to precipitate most of the lipids present in the yolks (2).

H_2O-extraction: One part of egg yolk was diluted with 9 parts of Milli Q™ water. After 6 hours of slow stirring at 4°C the lipids were precipitated by centrifugation (10 000 × g, 25 min, 4°C) and the resulting supernatant with IgY was collected.

PEG 6000 extraction: One part of egg yolk was diluted with 3 parts of 0.1 M sodium phosphate buffer, pH 7.5, and 1 part of 17.5% PEG in 0.1 M sodium phosphate buffer, pH 7.5, resulting in a final concentration of 3.5% PEG. After 20 min of slow stirring at room temperature, the lipids were precipitated by centrifugation in a table centrifuge (3000 rpm, 25 min, room temperture). The resulting supernatant with IgY was collected and filtered through a coarse filter paper to remove remaining particles (Munktell, 1F).

Before either of the extracts were applied to the HiTrap IgY Purification HP columns, potassium sulphate was added to a final concentration of 0.5 M. After salt addition, the pH was adjusted to 7.5 and the sample was filtered through a 0.45 µm filter.

Purification and analysis

HiTrap IgY Purification HP

Thiophilic adsorption is promoted by water-structuring salts (3). The optimal adsorption buffer for IgY purificaiton on the thiophilic adorption medium (2-mercaptopyridine coupled to Sepharose™ High Performance) was determined to be 0.5 M potassium sulphate in 20 mM sodium phosphate buffer.

Column:	HiTrap IgY Purification HP 5 ml (1.6 × 2.5 cm)
Sample:	50 ml H_2O-extracted egg yolk (corresponds to ¼ of an egg yolk)
Binding buffer:	20 mM sodium phosphate, 0.5 M potassium sulphate, pH 7.5
Elution buffer:	20 mM sodium phosphate, pH 7.5
Cleaning buffer:	20 mM sodium phosphate, 30% isopropanol, pH 7.5
Flow rate:	5 ml/min
System:	ÄKTAexplorer™ 10S

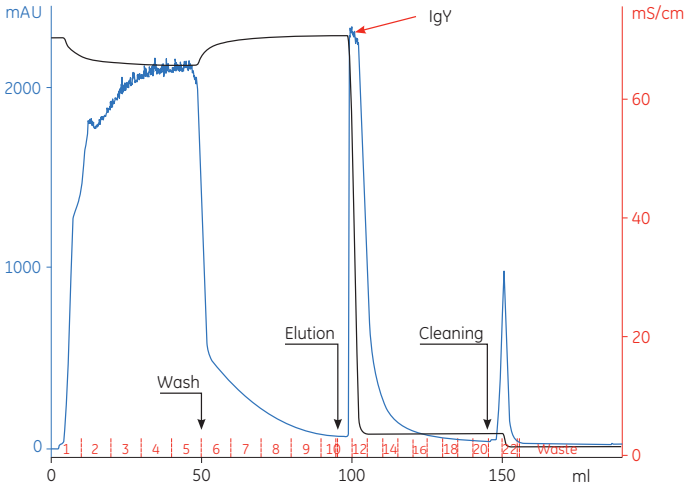
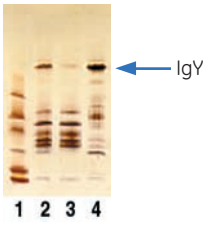


Fig 1. Purification of IgY from egg yolk after water extraction of lipids on HiTrap IgY Purification HP.

Table 1. Recovery of three specific IgY antibodies. Egg yolk was spiked with either α-fibrinogen or α-transferrin, while α-Hb was purified from eggs laid by hens immunized with Hb.

Antibody	Concentration of Ab in applied sample (µg/ml)	Amount of applied Ab present in flow through (%)	Amount of applied Ab present in eluate (%)
α-Hb	3.3	Not detectable	78
α-fibrinogen	193	13	70
α-transferrin	350	10	61



Lane 1: Low Molecular Markers
Lane 2: Egg yolk extract
Lane 3: Flow through = pool fractions 2 to 7
Lane 4: Eluate = pool fractions 12 to 16

Fig 2. Non-reducing SDS-PAGE analysis of fractions from HiTrap IgY Purification HP on PhastGel™ Gradient 10–15, silver stained.

Gel filtration

The pooled eluate fractions from HiTrap IgY Purification HP can be further purified by gel filtration on HiLoad™ 16/60 Superdex™ 200 prep grade. The purity of IgY in the eluate from HiTrap IgY Purification HP is about 70% as judged by analytical gel filtration on a Superdex 200, 10 × 300 mm column (Fig 3).

Column:	Superdex 200 10 × 300 mm column
Samples:	200 µl HiTrap IgY Purification HP eluate
Buffer:	50 mM sodium phosphate, 0.15 M NaCl, pH 7.5
Flow rate:	0.5 ml/min
System:	ÄKTAexplorer 10S

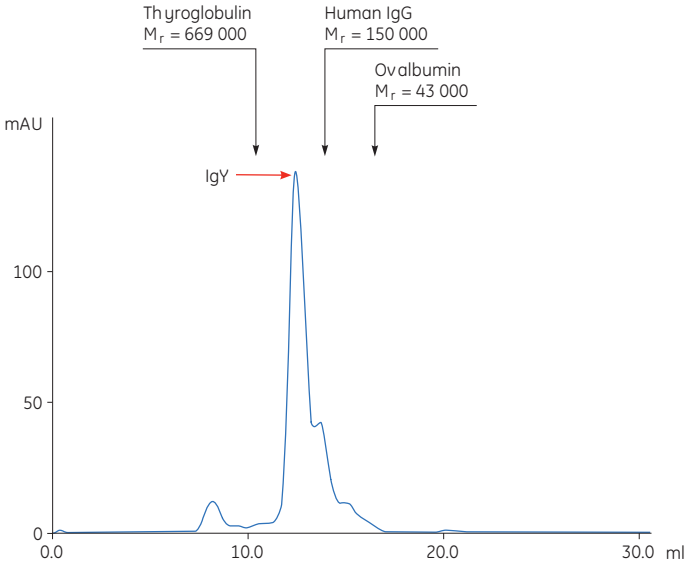


Fig 3. Gel filtration analysis of eluate from HiTrap IgY Purification HP.

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