The protocol was first described in Polson *et al*. 1980.

All steps should be performed using latex gloves.

1. The eggshell is carefully cracked and the yolk is transferred to a "yolk spoon" in order to remove as much egg white as possible.
2. The yolk is transferred to a filter paper and rolled to remove remaining egg white, then the yolk skin is cut with a lancet or a similar instrument (pipette tip). The yolk is poured into a 50 ml tube and the egg volume is registered (V1).
3. Twice the egg yolk volume of PBS is mixed with the yolk (∑V1+V2), thereafter 3.5 % PEG 6000 (in gram, pulverized) of the total volume is added and vortexed, followed by 10 min rolling on a rolling mixer. That step of the extraction procedure separates the suspension in two phases. One phase consists of "yolk solids and fatty substances" (original quotation of Polson et al. 1980) and a watery phase containing IgY and other proteins.
4. The tubes are centrifuged at 4°C for 20 min (10,000 rpm according to 13,000 x g, Heraeus Multifuge 3SR+, fixed angle rotor). The supernatant (V3) is poured through a folded filter and transferred to a new tube.
5. 8.5 % PEG 6000 in gram (calculated according to the new volume) are added to the tube, vortexed and rolled on a rolling mixer as in step 3.
6. Repeat step 4 with the difference that the supernatant is discarded.
7. The pellet is carefully dissolved in 1 ml PBS by means of a glass stick and the vortexer. PBS is added to a final volume of 10 ml (V4). The solution is mixed with 12 % PEG 6000 (w/v, 1.2 gram) and treated as in step 3 (vortex, rolling mixer).
8. Repeat step 6 and dissolve the pellet carefully in 800 μl PBS (glass stick and vortex). Wait for the air bubbles to disappear and then transfer (pipette) the extract to a dialysis capsule. Rinse the tube with 400 μl PBS and add the volume to the dialysis device (V5). (For preparation of dialysis devices and membranes see appendix.)
9. The extract is dialysed over night in 0.1 % saline (1,600 ml) and gently stirred by means of a magnetic stirrer. The next morning, the saline is replaced by PBS and dialysed for another three hours.
10. Thereafter the IgY-extract is pulled from the dialysis capsule by a pipette and transferred to 2ml tubes. The final volume is around 2 ml (V6).
11. It is advisable to store the samples in aliquots at -20°C (do not freeze the samples at -70°C).
12. The quality of the final preparations can be analysed by simple SDS-PAGE as described in JoVE protocol <http://www.jove.com/details.php?id=1916>

