

**Polyclonal**  
**IgY purification kit (Code : EPYK-FF KIT)**  
(applied to all bird egg yolks)

**KIT CONTENT**

(sufficient for 10 purifications of IgY from 1 egg yolk/each)

- **IgY Binding Gel** (Sephacrose™ fast flow) (Code : EPYK-FF) : 5 ml gel column.  
Binding capacity : > 20 mg IgY/ml wet gel.  
Purity : 98% by SDS-PAGE  
Maximum pressure : 3 bars (43 psi, 0.3 MPa).
- **IgY Binding Buffer** (Code : BBPY) 2x concentrated : 1,000 ml. Add 1,000 ml of distilled water to have a total of 2,000 ml before use.
- **IgY Elution Buffer** (Code : EBPY) 4x concentrated : 125 ml . Add 375 ml of distilled water to have a total of 500 ml before use.

(All the buffers used in the kit are aqueous solutions)

**INSTRUCTIONS FOR USE**  
**Purification of IgY**

1. Separate gently the yolk from the white of 1 hen egg and remove any residual egg white with a syringe or a Pasteur pipette connected to a low level vacuum (>15 mmHg).
2. Add such a crude egg yolk prepared in point 1 to 50 ml of IgY Binding Buffer (BBPY). Mix for 1-2 minutes to obtain a solution.
3. Centrifuge the falcon of IgY Binding Gel (Code: EPYK-FF). Discard the supernant from the gel cake.
4. Add the solution of egg yolk prepared in point 2 to the falcon containing the gel cake prepared in point 3. Mix gently the suspension for 30 minutes at 4°C.
5. Centrifuge at 3000 g and dicard the supernant from the gel cake..
6. Add 40 ml of IgY Binding Buffer (BBPY). Centrifuge and discard the supernatant. Repeat thrice more the same operation.
7. Add 50 ml of IgY Elution Buffer (Code : EBPY) and centrifuge. Collect the IgY supernant.
8. Assay the supernatant obtained as described in point 7, using the most appropriate system (SDS-PAGE, immunodiffusion, radioimmunoassay, Elisa...)

**REGENERATION OF THE IgY BINDING GEL**

**It is recommended to regenerate the gel after every 5 cycles of use.**

1. Wash the column with 10x volumes of NaOH 0.1M.
2. Wash the column with 10x volumes of distilled water.
3. Equilibrate the column 10x volumes of PBS (50 mM K<sub>2</sub>HPO<sub>4</sub>, 150mM NaCl) pH 7.4.
4. Store the column at 4°C in the presence of NaN<sub>3</sub> 0.1% (w/v).
5. For the next use, see INSTRUCTION FOR USE as described above.

**If you need sterile materials, the regeneration can be carried out as follows.**

**STERILE REGENERATION OF THE IgY BINDING GEL**

**(GEL SANITIZATION)**

**AFTER EVERY 5 CYCLES OF USE**

1. Wash 1 volume of gel column with 5 volumes of acetic acid 1 M.
2. Wash this column with 10 volumes of sterile distilled water.
3. Wash this column with 5 volumes of NaOH 1M.
4. Wash this column with 10 volumes of sterile distilled water.
5. Wash this column with 10 volumes of PBS (50 mM K<sub>2</sub>HPO<sub>4</sub>, 150mM NaCl) pH 7.4; NaN<sub>3</sub> 0.1%(w/v).
6. The sterile gel column is now ready to be re-used.