

Affinity Methodology in Biotechnology

# Polyclonal Rabbit IgG purification kit (Code : RbIKPG-FF KIT)

under physiological conditions, all steps

## Price: 300 EUR/KIT

## **KIT CONTENT**

## (sufficient for 8 purifications with 15 ml of rabbit serum /each)

- Rabbit IgG Binding Gel (Sepharose<sup>™</sup> fast flow) (Code : RbIKPG-FF) : 5 ml gel column. Binding capacity : approx. 20 mg rabbit IgG/ml wet gel. Purity : 90% by SDS-PAGE Maximum pressure : 3 bars (43 psi, 0.3 MPa). Gel life : approx. 50 cycles with routine regeneration.
- Rabbit IgG Binding Buffer (Code : BBRbPG) 2x concentrated : 1,000 ml (Add 1000 ml of distilled water before use)
- Rabbit IgG Elution Buffer (Code : EBRbPG) 4x concentrated : 125 ml (Add 375 ml of distilled water before use).
- Rabbit IgG Precipitating Agent (Code : PARbPG) : 8 x 1 sachet of sufficient quantity for precipitating all IgG from 15 ml of rabbit serum/each.

## **INSTRUCTIONS FOR USE**

- 1 Add with mild agitation 1 sachet of Precipitating Agent (PARbPG) to 15 ml of rabbit serum for 10 minutes. Stop the agitation and allows to stand for 30 minutes at 4°C. Centrifuge at 3000 g for 10 minutes. Discard the supernant from the pellet. Dissolve the pellet in 30 ml of rabbit IgG Binding Buffer (BBRbPG). Such a sample is ready to be loaded into the column.
- 2. Equilibrate the column (RbIKPG-FF) with 20 ml of rabbit IgG Binding Buffer (BBRbPG). Set the valve to get a flow rate of approx. 30 ml/hour.
- 3. Load the sample prepared in point 1 into the column prepared in point 2 at a flow rate of 30 ml/hour.
- 4. Wash the column with 200 ml of rabbit IgG Binding Buffer (BBRbG) at a flow rate of approx. 50 ml/hour.
  5. Elute the rabbit IgG with the rabbit IgG Elution Buffer (Code : EBRbPG) until the O.D. at 280nm of the
- eluent reaches the baseline level. Collect 10 x 5 ml fractions. Pool all protein containing fractions. 6. If you want an important concentration of rabbit IgG without loss of its content, use our "Concentraton Resin".
- 7. Assay the elution fractions obtained as described in point 5, using the most appropriate system (SDS-PAGE, immunodiffusion, radioimmunoassay, Elisa...)

## **REGENERATION OF THE RABBIT IGG 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 RABBIT IgG 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  $K_2$ 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.