

## Monoclonal Human IgG purification kit (Code : HIKG-FF KIT)

Price: 300 EUR/KIT

### KIT CONTENT

(sufficient for 8 purifications with 100 ml of human cell culture supernatant and/or ascites fluid/each)

- **Human IgG Binding Gel** (Sephacrose™ fast flow) (Code : HIKG-FF) : 5 ml gel column.  
Binding capacity : approx. 20 mg human 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.
- **Human IgG Binding Buffer** (Code : BBHG) 2x concentrated : 1,000 ml. Add 1000 ml of distilled water to have a total of 2000 ml before use.
- **Human IgG Elution Buffer** (Code : EBHG) 4x concentrated : 125 ml. Add 375 ml of distilled water to have a total of 500 ml before use.
- **Human IgG Precipitating Agent** (Code : PAHG) : 8 x 1 sachet of sufficient quantity for precipitating all IgG from 100 ml of cell culture supernatant and/or ascites fluid/each.

### INSTRUCTIONS FOR USE

1. Add with mild agitation 1 sachet of Precipitating Agent (PAHG) to 100 ml of human cell culture supernatant (and/or ascites fluid) for 15 minutes. Stop the agitation and allows to stand for 30 minutes at 4°C . Centrifuge at 3000 g for 10 minutes. Discard the supernatant from the pellet. Dissolve the pellet in 30 ml of human IgG Binding Buffer (BBHG). Such a sample is ready to be loaded into the column.
2. Equilibrate the column with 20 ml of human IgG Binding Buffer (BBHG).
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 human IgG Binding Buffer (BBHG).
5. Elute the human IgG with the human IgG Elution Buffer (Code : EBHG) until the O.D. at 280nm of the eluent reaches the baseline level. Collect 10 fractions of 5 ml elution volume.
6. **If you want an important concentration of human IgG without loss of its content**, use our **Protein concentration kit (Code: PC KIT)**.
7. Assay the elution fractions obtained as described in point 5, using the most appropriate system (SDS-PAGE, immunodiffusion, radioimmunoassay, Elisa...)

### REGENERATION OF THE HUMAN 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 HUMAN 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<sub>2</sub>HPO<sub>4</sub>, 150mM NaCl) pH 7.4; NaN<sub>3</sub> 0.1%(w/v).
1. The sterile gel column is now ready to be re-used.