

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

### INSTRUCTIONS FOR USE

1. Add with mild agitation 1 sachet of Precipitating Agent (PAHM) to 100 ml of cell culture supernatant (and/or ascites fluid) for 15 minutes. Stop the agitation and allow 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 mouse IgG Binding Buffer (BBHM). Such a sample is ready to be loaded into the column.
2. Pour the suspension of 5 ml of Human IgM Binding Gel (Code : HIKM-FF) into an appropriate column of your choice having 1 cm diameter of section. Then equilibrate the column with 20 ml of Human IgM Binding Buffer (BBHM). 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 human IgM Binding Buffer (BBHM) at a flow rate of approx. 50 ml/hour.
5. Elute the human IgM with the Human IgM Elution Buffer (Code : EBHM) 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 human IgM 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 IgM 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 IgM 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.
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2. 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).
3. The sterile gel column is now ready to be re-used.