

Monoclonal Rabbit IgM purification kit (Code : RbIKM-FF KIT)

Price: 300 EUR/KIT

KIT CONTENT

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

- **Rabbit IgM Binding Gel** (Sepharose™ fast flow) (Code : RbIKM-FF) : 5 ml gel column.
Binding capacity : approx. 10 mg rabbit 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.
- **Rabbit IgM Binding Buffer** (Code : BBRbM) 2x concentrated : 1,000 ml. Add 1000 ml of distilled water to have a total of 2000 ml before use.
- **Rabbit IgM Elution Buffer** (Code : EBRbM) 4x concentrated : 125 ml. Add 375 ml of distilled water to have a total of 500 ml before use..
- **Rabbit IgM Precipitating Agent** (Code : PARbM) : 8 x 1 sachet of sufficient quantity for precipitating all IgM from 100 ml of rabbit cell culture supernatant and/or ascites fluid/each.

INSTRUCTIONS FOR USE

1. Add with mild agitation 1 sachet of Precipitating Agent (PARbM) to 100 ml of 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 rabbit IgM Binding Buffer (BBRbM). Such a sample is ready to be loaded into the column.
2. Equilibrate the column (RbIKM-FF) with 20 ml of rabbit IgM Binding Buffer (BBRbM). 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 IgM Binding Buffer (BBRbM) at a flow rate of approx. 50 ml/hour.
5. Elute the rabbit IgM with the rabbit IgM Elution Buffer (Code : EBRbM) 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 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 RABBIT 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 K₂HPO₄, 150mM NaCl) pH 7.4.
4. Store the column at 4°C in the presence of NaN₃ 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 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.
4. Wash this column with 10 volumes of sterile distilled water.
5. Wash this column with 10 volumes of PBS (50 mM K₂HPO₄, 150mM NaCl) pH 7.4; NaN₃ 0.1%(w/v).
6. The sterile gel column is now ready to be re-used.