# Monoclonal
# Rat IgM purification kit (Code : RIKM-FF KIT)
**Price: 300 EUR/KIT**

## KIT CONTENT
**(sufficient for 8 purifications with 100 ml of rat cell culture supernatant and/or ascites fluid/each)**

- Rat IgM Binding Gel (Sepharose™ fast flow) (Code : RIKM-FF) : 5 ml gel column.
  Binding capacity : approx. 12 mg rat IgM/ml wet gel.
  Purity : 95% by SDS-PAGE
  Maximum pressure : 3 bars (43 psi, 0.3 MPa).
  Gel life : approx. 50 cycles with routine regeneration.
- Rat IgM Binding Buffer (Code : BBRM) 2x concentrated : 1000 ml. Add 1000 ml of distilled water to have a total of 2000 ml before use.
- Rat IgM Elution Buffer (Code : EBRM) 4x concentrated: 125 ml. Add 375 ml of distilled water to have a total of 500 ml before use..
- Rat IgM Precipitating Agent (Code : PARM) : 8 x 1 sachet of sufficient quantity for precipitating all IgM from 100 ml of rat cell culture supernatant and/or ascites fluid/each.

## INSTRUCTIONS FOR USE

1. Add with mild agitation 1 sachet of Precitating Agent (PARM) to 100 ml of rat 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 supernant from the pellet. Dissolve the pellet in 30 ml of Binding Buffer (BBRM). Such a sample is ready to be loaded into the column.
2. Equilibrate the column (RIKM-FF) with 20 ml of rat IgM Binding Buffer (BBRM). 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 rat IgM Binding Buffer (BBRM) at a flow rate of approx. 50 ml/hour.
5. Elute the rat IgM with the rat IgM Elution Buffer (Code : EBRM) 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 rat 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 RAT 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 $K_2HPO_4$, 150mM NaCl) pH 7.4.
4. Store the column at 4°C in the presence of $NaN_3$ 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 RAT 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 $K_2HPO_4$, 150mM NaCl) pH 7.4; $NaN_3$ 0.1%(w/v).
6. The sterile gel column is now ready to be re-used.