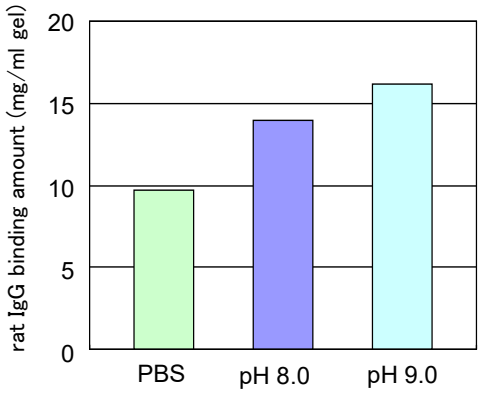
Ab-Capcher™: rat IgG purification
Effect of binding buffer pH

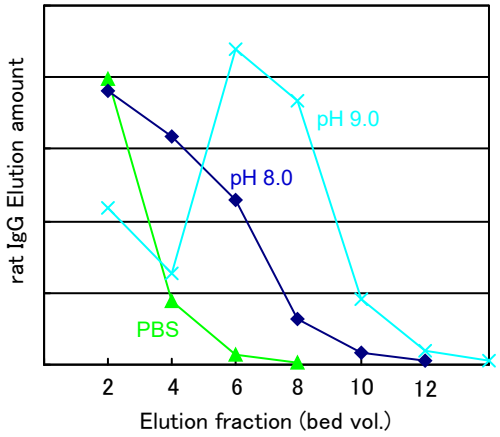
Purification flow chart

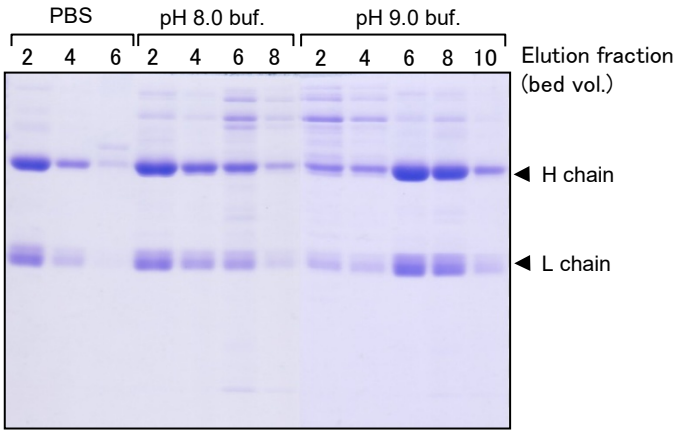
Rat serum
↓
50% ammonium sulfate precipitation fraction
↓
Dialysis (PBS)
↓
PBS or
1.5 M Glycine / 3M NaCl, pH8.0,
3-fold dilution with 1.5M Glycine / 3M NaCl,
pH 9.0
↓
Addition to Ab-Capcher™ gel (spin column)
↓
Shake (2 hours)
↓
Wash (binding buffer)
↓
Elute with 0.1 M Glycine-HCl (pH 2.8)
(Add 1 M Tris to neutralize)
↓
Pool 2 bed vols

Rat serum IgG binding amount



Analysis of IgG elution pattern





Sample volume: 2 μl; Gel: 12.5%; Stain: CBB

A 50% ammonium sulfate precipitate of rat serum was adjusted to three different pH values and bound to Ab-Capcher™. Following elution, yield and purity were determined. Increasing the pH of the binding buffer increased the yield of rat serum IgG, but also increased the amount of contaminants. However, it was found that high-purity rat IgG can be efficiently purified by pooling the IgG-rich fractions of the eluted fraction. In PBS, we pooled up to 4 bed vols. At pH 8, contaminants elute in bed vol fraction 6, so up to 4 bed vol. was pooled as in PBS. In the case of pH 9, contaminants elute up to bed vol 4, so 6 and 8 bed vol fractions were pooled. As a result, at pH 9, 16.1 mg/ml gel of rat IgG corresponding to 1.7 times that of PBS could be purified.

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