Application data No.043



Purification flow chart

oteNova

Mouse ascites ↓ 3-fold diluted with PBS ↓ Add to Ab-Capcher ExTra[™] ↓ Shake (25 °C, 1 hour) ↓ Wash (PBS)

Elute at different pH values

* Elution buffer: 0.1 M Glycine-HCl, pH 2.8 0.1 M Na citrate, pH 4 Containing 1 M arginine 0.1 M Na citrate, pH 5

Electrophoresis result

Gel: 15% (Tris-Glycine) Stain: CBB

Lane 1: mouse ascites Lane 2: pH 2.8 Eluate Lane 3: pH 4 Eluate Lane 4: pH 5 + Arg Eluate Lane 5: MW marker

IgG1 binding amount

Elution pH	Mouse IgG1 (mg/mL gel)
pH 2.8	14.2
рН 4	12.9
pH 5 + Arg	11.9



Purification of IgG1 at acidic pH may cause problems such as aggregation and decreased activity. The standard protocol of Ab-Capcher ExTra[™] recommends elution at pH 2.8, although it has been confirmed that mouse IgG can elute at higher pH. Therefore, we examined the role of elution pH in purifying mouse IgG1 from ascites with Ab-Capcher ExTra[™]. Normalizing to IgG1 recovery with elution at pH 2.8, we observed 91% recovery at pH 4 and about 83% recovery at pH 5 in buffer containing arginine*. SDS-PAGE analysis confirmed that high purity recovery could be obtained under any of the tested elution pH values. Thus, Ab-Capcher ExTra[™] permits elution at high pH to purify mouse IgG1 from ascites with high yield and purity. * Arakawa, et al. "Elution of antibodies from a Protein A column by aqueous arginine solutions" Protein Expr. Puri. 36 (2004) 244-248.

Protenova Co., Ltd. 〒769-2604 1488 Nishimura, Higashikagawa City, Kagawa Prefecture TEL 0879-49-0702 / FAX 0879-49-0703 Home page http://protenova.com