

Purification of rabbit IgG from rabbit anti-glucosylceramide (RAS_0010)

Preparation of column:

Fill 2.5 ml Protein A Sepharose 4FF in appropriate column

Wash with 50 ml distilled water at a flow rate of 1.0 ml/min.

Wash with 50 ml 10 mM Tris-HCl pH 8.0 at a flow rate of 0.5 ml/min

Loading the column:

dialyze 5 ml of RAS_0010 against 10 mM Tris-HCl pH 8.0

circulate dialyzed serum three times through column at a flow rate of 0.1 ml/min

wash with 50 ml 10 mM Tris-HCl pH 8.0 at a flow rate of 0.5 ml/min

Elution:

elute with 30 ml 0.1 M glycine-HCl pH 3.0 at a flow rate of 0.5 ml/min

collect fractions of 1 ml

bring immediately to appr. pH 5.0 with NaHCO_3

test fractions by dot blot

pool appropriate fractions

determine protein concentration

add 0.02% NaN_3 and store at 4 °C or freeze at -20 °C

Regenerate column:

wash with 50 ml 0.1 M glycine-HCl pH 3.0 at a flow rate of 1.0 ml/min.

wash with 50 ml 0.1 M glycine-HCl pH 2.3 at a flow rate of 1.0 ml/min.

wash with 50 ml distilled water at a flow rate of 1.0 ml/min.

wash with 50 ml PBS pH 7.2 + 0.02% NaN_3 at a flow rate of 1.0 ml/min.

store at 4 °C

In numerous purifications the yield was between 23 and 29 mg