**Procedure of column equilibration and sample depletion using ProteoExtract Albumin/IgG removal kit**

Procedures were mainly showed in the product protocol, however, several steps have been modified due to the experimental needs, e.g. the evaporation time and final concentration of the protein in the sample.

**The preparation steps:**

1. Blue cap was removed from a proteoextract Albumin/IgG Removal column. The column was inverted on a tissue paper to allow for fast removal of column storage buffer (for 5 mins)
2. The lower tip of the column was removed and placed in a 15 ml tube
3. 850 µL of the binding buffer was added to the column and allowed passing the resin bed by gravity flow (8 mins)
4. 40 µl of rat plasma was diluted with 360 µl of biding buffer
5. The collection tube was discard
6. Equilibrated column in a fresh 15 ml collection tube was placed before applying the diluted sample
7. The diluted sample was applied to the column. The sample was allowed to pass through the resin bed by gravity-flow (6 mins).
8. 600 µl of biding buffer was added to wash the column by gravity-flow. It was collected in a same collection tube when it was flow-through.

Procedure of eluted bound protein

1. Laemmli buffer [50 ml Laemmli buffer containing 0.3785 g Tris base, (62.4 mM); 1.0279 g SDS (2%)f; buffer 1 containing 9.92 ml glycerol (25 % W/V) or buffer 2 which containing 3.97 ml (10% W/V), pH 6.8] (1 ml) was added into a 2 ml top pierced tube. The tube was then put in a rack and boiled for 5 minutes in a beaker with boiling ddH2O.
2. The tube was taken out of the water bath and cooled at room temperature for 1 minute before loaded onto the column.
3. 850 µl of Laemmli buffer was added on the column with a new 15 ml collection tube. Allowed to pass through by gravity-flow for 15 minutes.
4. Step 3 was repeat for further washing to elute the bound protein.