FG winds

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Immunoprecipitation (1)

Summary

FG beads® is suitable for various research fields including the chemical biology and also demonstrate superior performance in immunoprecipitation.

Method for antibody immobilization on FG beads® :
There are mainly two methods: one is to bind antibody covalently to beads by using activated functional groups such as NHS beads and the other is to bind antibody to beads by Protein A, Protein G, etc.
Both beads have different advantages. NHS beads can reduce the amount of detached antibody in elution step and reduce non-specific background than use of Protein A and Protein G due to direct binding. On the other hand, Protein A and Protein G beads are easy to use / bind the antibody with its strong affinity against IgGs.

We here show the advantages of our Protein G beads with higher recovery rate than other competitors by two different experiments.

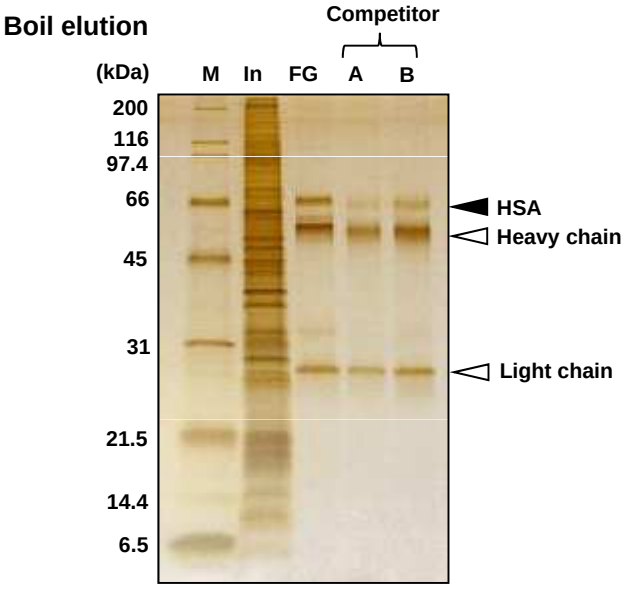
Result (1)

1. Experiment with the same amount of beads

0.1mg of FG beads® have the capacity to bind antibody by 10ug or more, and therefore can fully bind the antibody required for general immunoprecipitation.

We compared the recovery amounts of antigens for the beads (including FG beads®) of three companies, using 0.1mg of the beads and 5ug of Anti-HSA.

The results show that FG beads® have the largest recovery amount.



Condition

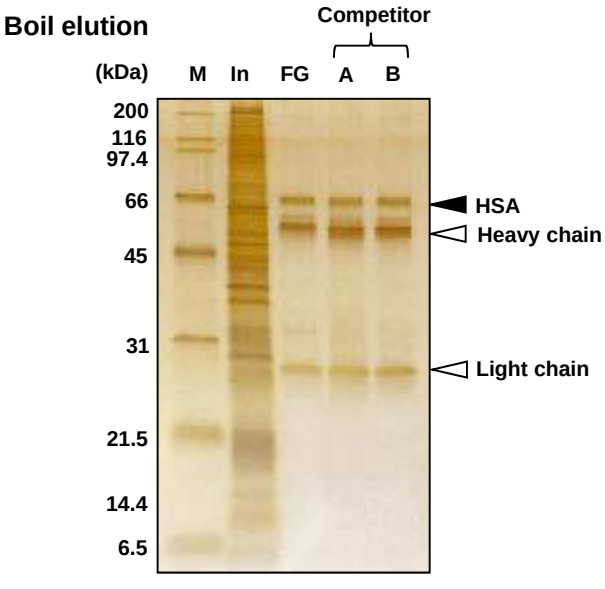
	Beads	Antibody
FG beads®	0.1mg	5ug
A	0.1mg	5ug
B	0.1mg	5ug

2. Experiment to obtain the same recovery amount of antigens

We performed immunoprecipitation to verify how much the beads of each company is required respectively to recover the same amount of antigens when 5ug of Anti-HSA is used.

The results show that the recovery amount of antigens for each company becomes the same under the conditions shown below.

It is, therefore, confirmed that the amount of FG beads® required for the immunoprecipitation is enough by 1/15 of that of competitor A and 1/2.5 of that of competitor B.



Condition

	Beads	Antibody
FG beads®	0.1mg	5ug
A	1.5mg	5ug
B	0.25mg	5ug

※The above experiments were performed based on protocols recommended by each company.

Materials and method

■Materials

1. Anti-HSA (Human Serum Albumin) (from Hytest)
2. HSA (from nacalai tesque)
3. HeLa cell extracts (cytosolic fraction) – 3mg/ml
4. Protein G beads
5. PBS(-) (137mM NaCl, 2.7mM KCl, 8.1mM $Na_2HPO_4 \cdot 12H_2O$, 1.5mM KH_2PO_4)
6. Wash Buffer A (10mM HEPES-NaOH(pH7.9), 50mM KCl, 0.2mM EDTA, 10%(v/v) glycerol)
7. Wash Buffer B (20mM HEPES-NaOH(pH7.9), 150mM KCl, 1mM $MgCl_2$, 0.2mM $CaCl_2$, 0.2mM EDTA, 10%(v/v) glycerol, 0.1% NP-40, 0.2mM PMSF)
8. Elution Buffer (0.1M glycine-HCl pH2.5)
9. Neutralization Buffer (1M Tris-HCl pH9.0)
10. SDS sample buffer (62.5mM Tris-HCl (pH6.8), 0.005% BPB, 2% SDS, 10% glycerol, 5% 2-mercapto ethanol)

■FG beads® information

Product name	Protein G beads
Product number	TAS8848N1173
Storage temperature	2-8ºC
Storage buffer	10mM HEPES(pH7.9), 50mM KCl, 1mM EDTA, 10%glycerol
Size of beads	190nm ± 20nm
IgG binding capacity	>100ug mouse IgG /mg of beads

■Method 1 (Binding Antibody)

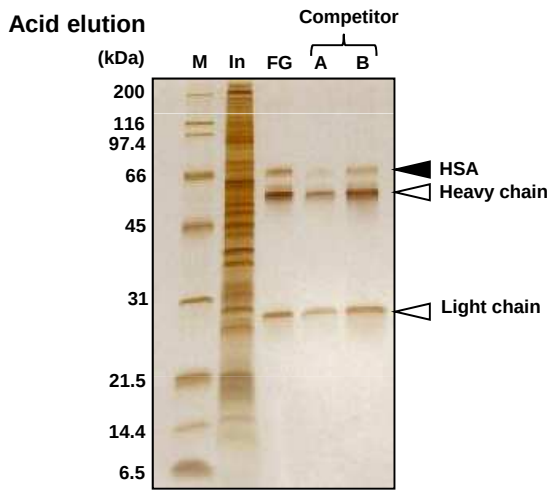
1. Wash
Transfer each amount of beads to a tube.
Wash beads with 200ul PBS(-) 2 times at 4ºC.
2. Bind Antibody
Add antibody (5ug) diluted in 200ul PBS(-) to beads.
3. Reaction
Mix for 30min at room temperature.
4. Wash
Wash antibody binding beads with Wash buffer A 2 times at 4ºC.

■Method 2 (Immunoprecipitation)

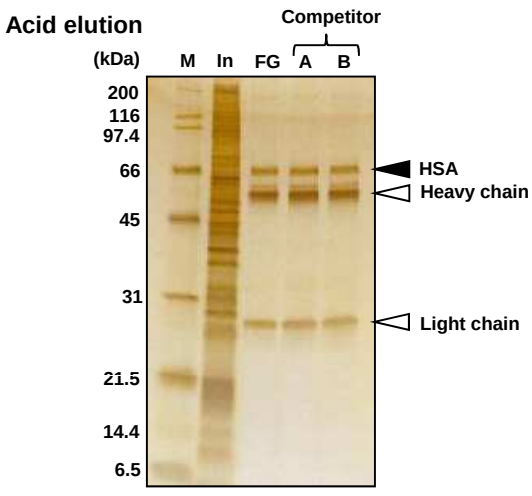
1. Add sample solution
Add 200ul HeLa cell extracts containing HSA to the antibody binding beads.
2. Reaction
Resuspend beads and incubate with rotation for 120min at 4ºC.
3. Wash
Separate magnetically and remove supernatant.
Wash beads with Wash buffer B 3 times at 4ºC.
Transfer 100ul resuspended solution to new tube to perform 2 elution pattern.
Separate magnetically and remove supernatant.
4. Elution
Acid elution
Add 28ul Elution buffer and resusupend beads.
Stand for 5min at 4ºC, separate magnetically.
Transfer the supernatant to new clean tube and add 2ul Neutralization buffer, and 10ul 4×SDS sample buffer.
Boil elution
Add 40ul 1×SDS sample buffer and resuspend beads.
Boil for 5min and remove the beads.
5. Analyze the samples by SDS-PAGE and silver staining

Result (2)

1. Experiment with the same amount of beads



2. Experiment to obtain the same recovery amount of antigens



Acid elution samples show similar band pattern to Boil elution samples.