

High Performance Magnetic Nanoparticles



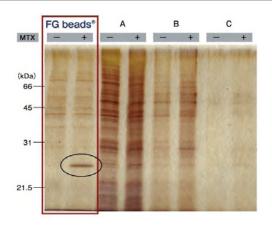
The FG (Ferrite-glycidyl methacrylate) beads developed by Tokyo Institute of Technology consist of 200 nm-diameter ferrite nanoparticles coated firmly with a polymer layer.

The FG Beads are used as carriers for affinity purification of target proteins. ¹⁾

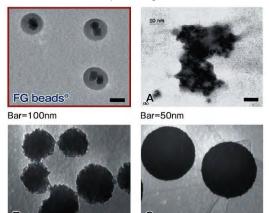
Features

- Extremely low non-specific binding Poly-GMA (Glycidyl methacrylate) coated magnetic nanoparticles.
- Excellent recovery of target proteins 200 nm particles have a large surface area and a high dispersibility.
- High stability in organic solvents Various compounds can be immobilized on the beads.





Affinity purification of MTX binding proteins Affinity purification of MTX binding proteins Immobilization of MTX on commercial magnetic beads was done in the same manner as in the case of FG beads. Electric microscope images



Target Protein

Ligand

Ferrite particles

Polymer layer

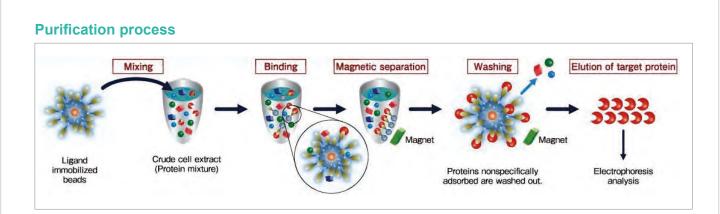
Linker

Bar=100nm

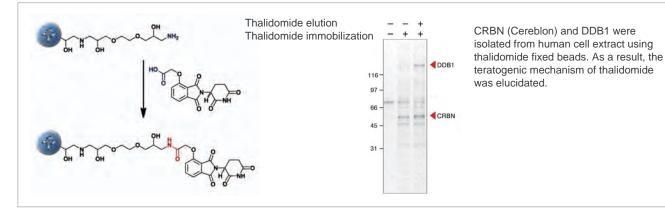
Bar=500nm

Linkers and Functional Groups

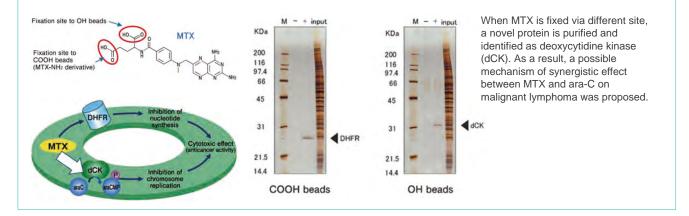
Linker and Function Group		Ligands to be fixed	Linker and Function Group		Ligands to be fixed
Plain beads		R-NH ₂ (Amino group) R-SH (Thiol group)	Streptavidin		Biotinylated compounds
Linker beads (epoxy beads)		R-OH (Hydroxy group)	NeutrAvidin™		Biotinylated compounds
NH ₂ beads	H H OH NH2 OH	R-COOH (Carboxy group)	Protein A		IgG
COOH beads	н он	R-NH ₂ , R-NHR' (Amino group)	Protein G		lgG
NHS beads	" OH H OH OH OH H OF NO NG			он о	
Azide beads		Alkynes			



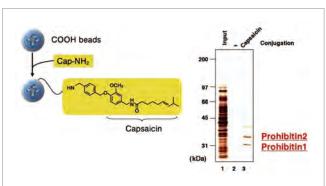
Purification of target protein of Thalidomide (elucidation of the teratogenic mechanism)³⁾



Purification of novel target protein of MTX (methotrexate) ⁴⁾

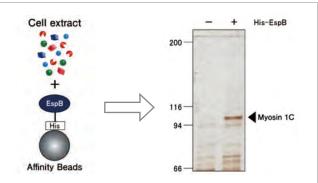


Purification of target protein of Capsaicin⁵⁾



Prohibitin 1 and prohibitin 2 were isolated from human myeloid leukemia NB4 cell extract using capsaicin derivative (Cap-NH₂) fixed beads. As a result, the apoptosis induction mechanism of capsaicin was elucidated.

Elucidation of the mechanism of enteropathogenic E. coli infection ⁶⁾



EspB is a protein of enteropathogenic E. coli (EPEC) essential for infection in humans. Myosin was isolated from human cell extract using EspB fixed beads. As a result, the mechanism of EPEC infection was elucidated.

Protein A / Protein G beads

- High recovery

- High purity

IgG binding capacity

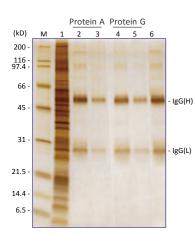
- more than twice the amount of a competitor. Extremely low non-specific adsorption.

- Quick processing 30 minutes for IgG binding

Applications

- IgG purification
- Immunoprecipitation (IP)
- Chromatin Immunoprecipitation (ChIP)
- Protein separation

IgG Purification



Sample: 1. HeLa Extract + IgG 2. FG beads (Protein A) 3. Competitor (Protein A)

4. FG beads (Protein G) 5. Competitor (Protein G)

6. Input IgG Detection: Silver Staining

We compared the binding capacity of FG beads to capture IgG in HeLa cell extracts with the beads of a competitor. FG beads captured larger amounts of IgG than the competitor's beads.

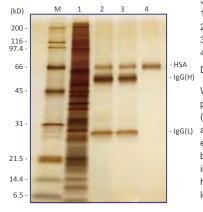
- 1. Add 10 μg of IgG into 200 μg of HeLa cell extracts (200 $\mu l).$
- 2. Add 300 µg of each beads to the HeLa cell extracts.
- 3. React for 10 min at 4°C and separate beads from the HeLa cell extract.
- 4. Elute bound IgG by adding Glycine-HCl.

Streptavidin / NeutrAvidin beads

- High recovery

Biotin binding capacity - more than twice the amount of a competitor.

Immunoprecipitation



Sample:

1. HeLa Extract + HSA 2 FG beads (Protein A)

- 3. FG beads (Protein G)
- 4. Input IgG

Detection: Silver Staining

- We checked the performance of FG beads (Protein A and Protein G) in
- IgG(L) an immunoprecipitation experiment. By using FG beads, antigen HSA was immunoprecipitated with high recovery and extremely low non-specific adsorption.

1. Immobilize anti-Human Serum Albumin antibody on FG beads.

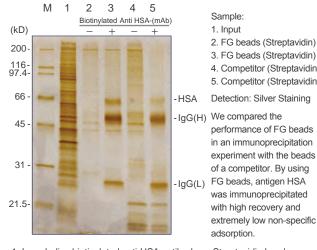
- 2. Add 400 ng of HSA into 200 μg of HeLa cell extracts (200 $\mu l).$

- High purity

Applications • Immunoprecipitation (IP)

- · Chromatin Immunoprecipitation (ChIP)
- · Cell separation
- · Affinity purification of drug target proteins

Immunoprecipitation

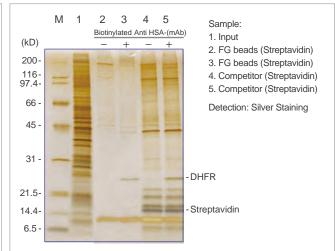


- 3. FG beads (Streptavidin)
- 4. Competitor (Streptavidin)
- 5. Competitor (Streptavidin)
- Detection: Silver Staining

IgG(H) We compared the performance of FG beads in an immunoprecipitation experiment with the beads of a competitor. By using IgG(L) FG beads, antigen HSA was immunoprecipitated with high recovery and extremely low non-specific

- 1. Immobolize biotinylated anti-HSA antibody on Streptavidin beads.
- 2. Add 1 mg of HSA into 600 µg of HeLa cell extracts (200 µl).
- 3. Add 0.5 mg of each beads to the HeLa cell extracts.
- 4. React for 60 min at 4°C and separate beads from the HeLa cell extract.
- 5. Elute bound IgG and HSA by adding Glycine-HCI.

Affinity purification of drug target protein



We compared the performance of biotinylated drug MTX (Methotrexate) immbilized FG beads with the beads of a competitor in a target protein purification expertiment. By using FG beads, MTX target protein DHFR was purified with extremely low non-specific adsorption.

- 1. Immobolize biotinylated MTX on Streptavidin beads.
- 2. Add 0.5 mg of each beads into 600 μg of HeLa cell extracts (200 $\mu l).$
- 3. React for 120 min at 4°C and separate beads from the HeLa cell extract.
- 4. Elute bound DHFR by adding elution buffer.

- 3. Add 0.1 mg of each beads to HeLa cell extracts.
- 4. React for 120 min at 4°C and separate beads from the HeLa cell extract.
- 5. Elute bound IgG and HSA by adding Glycine-HCI.
- Extremely low non-specific adsorption.

Magnetic Stand



- Quick cooling down

The magnetic stand made of metal can quickly cool down samples on ice. you can experiment without protein denaturation.

- High speed separation

The magnetic stand separates magnetic nanoparticles in shorter time than competitors because shape and placement of magnets are well designed.

Reference

- 1) S. Sakamoto et al., Chem. Rec. 9 (2009) 66
- 2) K. Nishio et al., Colloids Surfaces. B. 64 (2008) 162
- 3) T Ito et al., Science 327 (2010) 1345
- 4) H. Uga et al., Mol. Pharmacol. 70 (2006) 1832
- 5) C. Kuramori et al., Biochem. Biophys. Rec. Commun. 379 (2009) 519
- 6) Y. lizumi et al., Cell Host & Microbe. 2 (2007) 383

Ordering Information

Product Name	Storage	Product No.	PKG Size
Plain beads	R	TAS8848N1010	10 mg
Linker beads (epoxy beads)	R	TAS8848N1110	5 mg
NH ₂ beads	R	TAS8848N1130	5 mg
COOH beads	R	TAS8848N1140	5 mg
NHS beads	F	TAS8848N1141	5 mg
Azide beads	R	TAS8848N1160	5 mg
Streptavidin beads	R	TAS8848N1170	5 mg
NeutrAvidin [™] beads	R	TAS8848N1171	5 mg
Protein A beads	R	TAS8848N1172	5 mg
Protein G beads	R	TAS8848N1173	5 mg
Othres			
Magnetic stand (for 1.5 ml tube)	RT	TA4899N12	1 ea
Magnetic stand (for 15 ml tube)	RT	TA4899N20	1 ea
Magnetic stand (for 50 ml tube)	RT	TA4899N30	1 ea
MTX derivatives	R	TAS8849N101	0.1 mg



(Storage) RT: Room Temperature R: Refrigerator F: Freezer NeutrAvidin™ is a tragemark of Thermo Fisher Scientific, Inc. and its subsidiaries.

For research use only, not intended for diagnostic or drug use.

NACALAI TESQUE, INC.

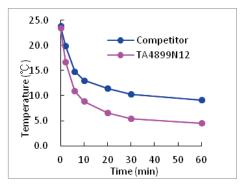
 Nijo Karasuma, Nakagyo-ku, Kyoto 604-0855 JAPAN

 TEL
 : +81-(0)75-251-1730

 FAX
 : +81-(0)75-251-1763

 Website
 : www.nacalai.com

 E-mail
 : info.intl@nacalai.com



Comparison of cooling speed of samples