PRODUCT DATA SHEET

Flag-Catch-Mag™ Anti-Flag alpaca nanobody conjugated Magnetic Beads

This product is only for research applications, not for diagnostic or therapeutic use.

Catalog Number: AT1770

Size: 500 µL (10mg/mL), 1 mL (10mg/mL)

Store at: +4°C, do not freeze

Background

Anti-FLAG Magnetic beads is an alpaca single domain antibody that is covalently attached to magnetic beads by hydrazide linkage. The antibody binds FLAG epitope at the N-terminal, Met-N-terminal, C-terminal and internal locations of fusion proteins.

Description:	Flag-Catch-Mag™ Anti- Flag tag nanobody (from alpaca VHH, Single Domain antibody) conjugated Magnetic Beads
Form of product:	Magnetic beads Conjugated anti-Flag nanobody, the product is supplied in 10 mM PBS, pH 7.4, and 0.02% (w/v) sodium azide as preservative.
Bead size:	~ 40 µm
Specificity:	This antibody detects FLAG-tagged proteins exogenously expressed in cells or E. coli. This antibody also detects 3*Flag-tagged proteins.
Epitope tag	DYKDDDDK
Reactivity:	Grab transfected FLAG fusion proteins expressed in E. coli, yeast, insect cell, mammalian cell, or in vitro transcription/translation systems. All systems expressing FLAG fusion protein would be suitable.
Tested applications:	Immunoprecipitation (IP), Co-Immunoprecipitation (Co-IP), Chromatin Immunoprecipitation (ChIP) RNA Binding Protein Immunoprecipitation (RIP) Enzyme assays Mass spectrometry Affinity purification In IP/CoIP, 25 µL of beads suspension for ~500-1000 µL of crude total cell lysate solution. Optimal dilutions/concentrations should be determined by the end user.
Binding capacity:	25 µg of FLAG fusion protein (~26 kDa) can be precipitated with 25 µL of beads suspension.
Host:	Alpaca
Clonality:	Monoclonal, nanobody
Isotype:	Alpaca single domain antibody (nanobody, VHH)
Immunogen:	This nanobody is produced from alpaca with DYKDDDDK peptide.
Storage instruction:	Store at +4°C and **do not freeze**.
Expiration Date:	Expires one year from date of receipt when stored as instructed

Attention: Magnetic Beads should be resuspended well before used in IP, Co-IP or ChIP.

Figure 1. A schematic representation of benefits (Alpaca Antibody Advantage)

- No interfering of traditional antibody heavy and light chains in your downstream WB and mass spectrometry analysis
- One step immunoprecipitation
- Highly specific binding
- Low background
- High affinity
- High stability
- Easy elution of native proteins

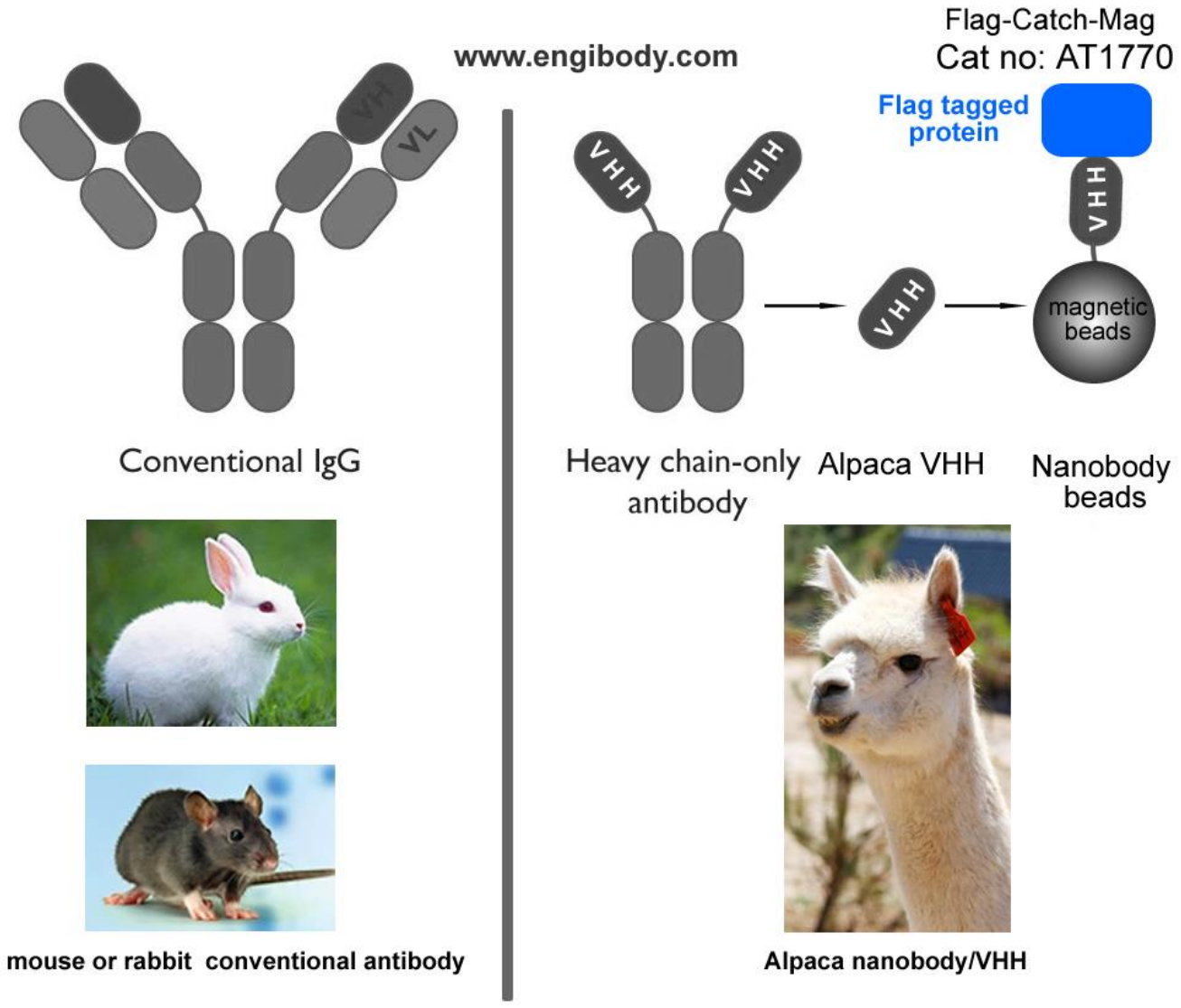
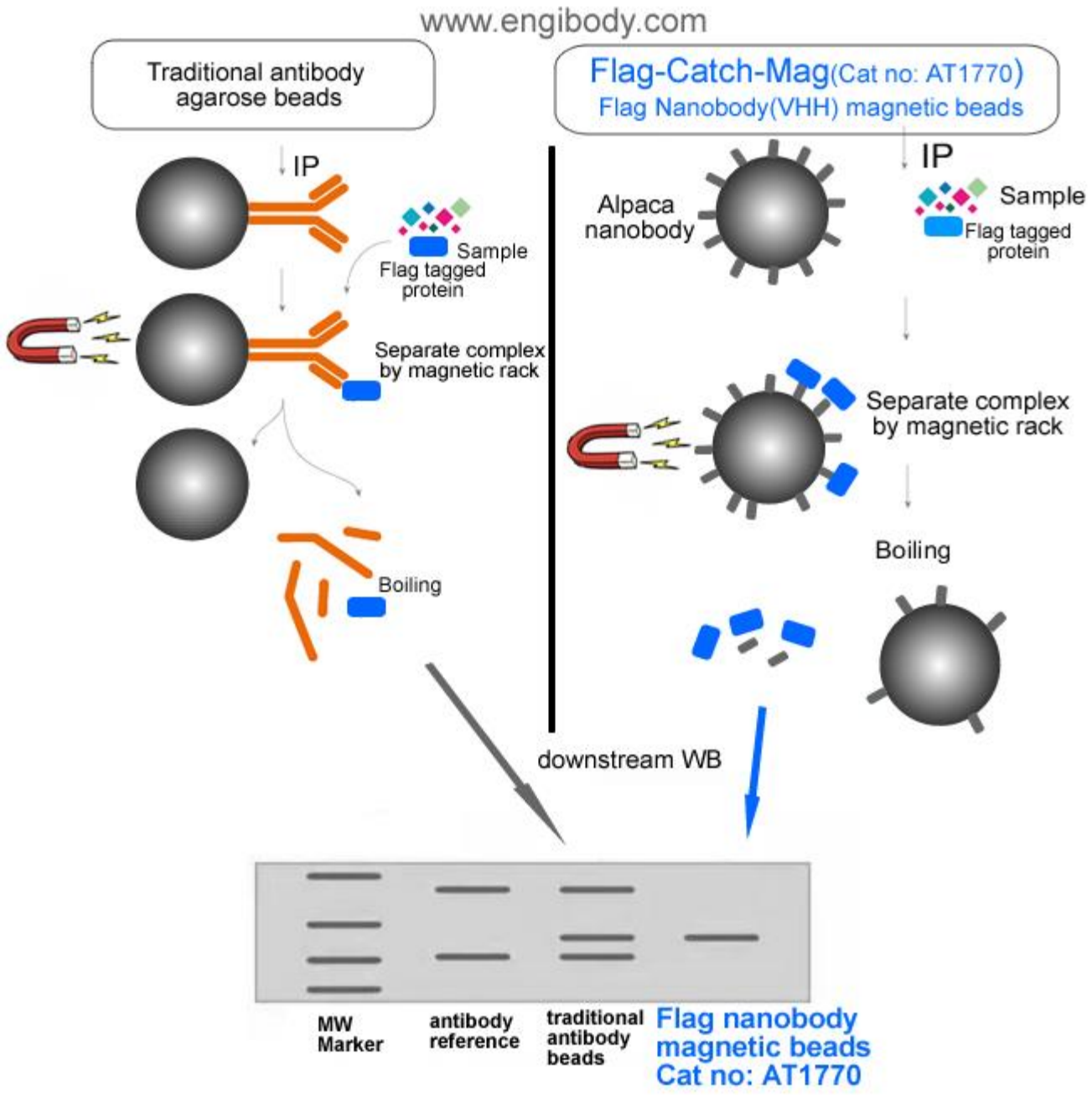
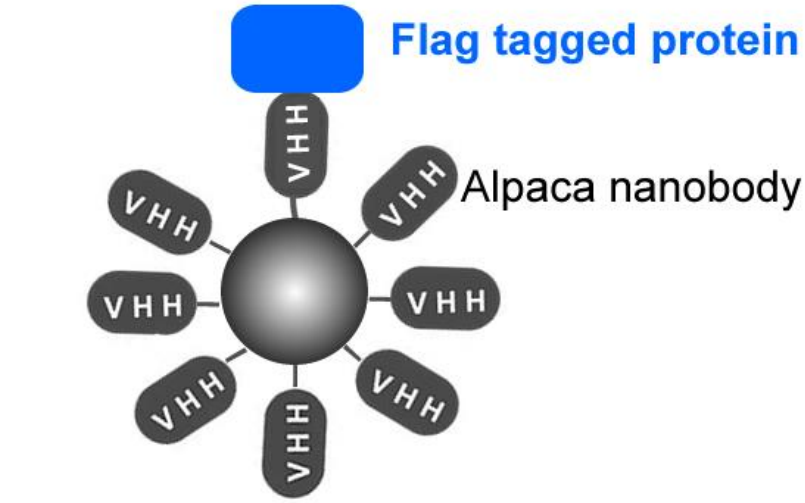


Figure 2. A schematic representation of benefits (no interfering of traditional antibody heavy and light chains)



Attention: Magnetic Beads should be resuspended well before used in IP, Co-IP and protein purification.

Structure Image



Protocol for Immunoprecipitation (IP/CoIP) of Flag-Fusion Proteins from Mammalian Cell Lysate (For soluble cytoplasmic protein)

Solutions and Reagents

1X Cell Lysis Buffer: 50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100. Recommend adding 1 mM PMSF before use.

1X Wash Buffer: TBS (50 mM Tris HCl, 150 mM NaCl, pH 7.5)

5X SDS Sample Loading Buffer

Procedure

Attention:

1. If your target protein is soluble nuclear protein in nucleus (Excluding nuclear membrane integrated protein and DNA-binding protein), please use NE-PER Nuclear and Cytoplasmic Extraction kit (Thermo, Cat no:78833) to extract nuclear protein. Or please use RIPA lysis buffer to lyse the cell, and add PIC, 1mM PMSF, 2.5mM Mgcl2 and 1mg/ml DNase I.

2. It is required to reserve 50 µl of protein extract for each sample as the INPUT control, which is directly used in the downstream WB experiment.

1. Preparing Cell Lysates

1. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS. Remove PBS and add 0.5 mL-1mL 1X ice-cold cell lysis buffer (added Protease Inhibitor Cocktail and PMSF) to each plate (10 cm, 10^6-10^7 cells) and incubate the plates on ice for 30-40 minutes with rotation at 4°C.
2. Scrape lysed cells off the plates and transfer to microcentrifuge tubes. Keep on ice.
3. Sonicate samples on ice three times for 5 seconds each (optional step).
4. Microcentrifuge for 10 minutes at 4°C, 14,000 x g, and transfer the supernatant to a new tube. If necessary, lysate can be stored at –80°C.

2. Equilibrate Beads

1. Resuspend the beads by inverting the product tube or gently pipetting up and down. Do not Vortex the beads.
2. Pipette 25 µL bead slurry into an EP tube (1.5 mL)，then add 1mL ice-cold wash buffer. Shake gently by your hand for 1-2 minutes. Do not vortex the beads.
3. Magnetic separation for 60 seconds.
4. Discard supernatant and repeat wash three times.

3. Immunoprecipitation

1. Take 500 µL cell lysate and add 25 µL of the antibody conjugated magnetic beads suspension, incubate with rotation for 1-3 hours at 4°C or overnight at 4°C.

4. Wash the protein-nanobody-beads complex

NOTE: if Co-IP interacting proteins are researched, please reduce the number of washes, and lower the ionic strength of the wash buffer.

Add 1.0 mL of Wash Buffer and suspend the beads complex, magnetic separation, then discard the supernatant. Repeat above steps at least four times.

5. Elution for Downstream Analysis

Elution of the FLAG fusion proteins - Three elution methods are recommended according to protein characteristics or further usage:

Option A: Native Elution

Elution under acidic conditions with 0.2 M glycine-HCl (pH 2.5). This is a fast and efficient elution method. Neutralization of the eluted proteins with neutralizing buffer (1 M Tris, pH 10.4) may help preserve its activity. Neutralizing buffer needs to be placed in the collection tube in advance.

Attention: It is necessary to make a preliminary experiment in advance to determine how much neutralization buffer is needed to neutralize glycine-HCl

Option B: Denaturing Elution for SDS-PAGE

Elution with sample loading buffer under denaturing conditions for gel electrophoresis and immunoblotting.

Option C: Protein elution under native conditions by competition with 3xFLAG peptide. The elution efficiency is very high using this method.

Option A: Elution with 0.2 M Glycine-HCl (pH 2.5) - The procedure should be performed at room temperature. Note：Do not leave the beads in this buffer more than 20 minutes.

1. Add 50 μL-100 μL of 0.2 M Glycine-HCl (pH 2.5), to each sample and control beads complexes.

2. Incubate the samples and controls with constantly pipette up and down for 1-3 minutes at room temperature. (Note: Do not turn the tube upside down to prevent the complex from sticking to the tube wall)

3. Place tube in the appropriate magnetic separator to collect the beads. Transfer the supernatants to fresh tubes containing about 5 μL of neutralizing buffer. Then use a pH-indicator paper (pH 6.4 - 8.0) to make sure the pH is 7.4. Be careful not to transfer any beads.

4. Repeat steps 1 – 3 in order to improve elution efficiency, pooling eluates in same tube or collect eluate through different tubes.

5. For immediate use, store the eluates at 2-8 °C. Store at –20 °C for long term storage.

Option B: Elution with SDS-PAGE Sample Loading Buffer

1. Resuspend each sample with 30 μL 1X SDS sample loading buffer (6 μL 5X SDS sample loading buffer can be added into 24 μL cell lysis buffer). Vortex.

2. Boil the sample and control tubes for 10 minutes at 95 – 100°C.

3. Place tubes in the magnetic separator to collect the beads. Transfer the supernatants to fresh tubes. The samples and controls are ready for loading on SDS-PAGE and immunoblotting using anti-flag or specific antibodies against the fusion protein.

Option C: Protein elution under native conditions by competition with 3xFLAG peptide. The elution efficiency is very high using this method.

1. Prepare 3X FLAG elution solution.
Prepare 3X FLAG peptide stock solution (5 mg/mL).
Dissolve 3X FLAG peptide (ENGIBODY, Cat. No: AT1968) in TBS (50mM Tris HCL, 150 mM NaCl, pH7.4) to a final concentration of 5 mg/mL. For extended storage after reconstitution, store at –20 °C in with 50% glycerol. Avoid repeated freeze-thaw.

Prepare 3X FLAG peptide working solution (1 mg/mL).
Dilute 5-fold with TBS to prepare a 3X FLAG peptide working solution containing 1 mg/mL of 3X FLAG peptide.

2. Add 100 μL of 3X FLAG elution working solution to each sample and control beads.

3. Incubate the samples and controls with gentle shaking for 30-60 minutes at 2–8 °C.

4. Separation by magnetic rack. Transfer the supernatants to fresh test tubes. Be careful not to transfer any beads.

5. For immediate use, store the supernatants at 2–8 °C. Store at –20 °C for long term storage.

Related products

Product	Cat. no.
Anti-Myc tag Mouse mAb	AT0023
Anti-HA tag Mouse mAb	AT0024
Anti-His tag Mouse mAb	AT0025
Anti-GST tag Mouse mAb	AT0027
Anti-GFP tag Mouse mAb	AT0028
Anti-V5 tag Mouse mAb	AT0026
Anti-RFP tag Mouse mAb	AT0038
Protein A/G Agarose Beads	IF0001
Protein A/G Magnetic Beads	IF0002
RFP-Catch-Aga™ Anti-RFP tag nanobody (VHH) conjugated Agarose Beads	AT1769
mCherry-Catch-Aga™ Anti-mCherry tag nanobody (VHH) conjugated Agarose Beads	AT1795
GFP-Catch-Aga™ Anti-GFP tag nanobody (VHH) conjugated Agarose Beads	AT1765
Flag-Catch-Aga™ Anti-DYKDDDDK (Flag) tag nanobody Conjugated Agarose Beads	AT1762
HA-Catch-Aga™ Anti-HA tag nanobody Conjugated Agarose Beads	AT1763
Myc-Catch-Aga™ Anti-Myc tag nanobody conjugated Agarose Beads	AT1764
Flag-Catch-Mag™ Anti-DYKDDDDK (FLAG) tag nanobody conjugated Magnetic Beads	AT1770
HA-Catch-Mag™ Anti-HA tag nanobody conjugated Magnetic Beads	AT1771
Myc-Catch-Mag™ Anti-Myc tag nanobody conjugated Magnetic Beads	AT1772
GFP-Catch-Mag™ Anti-GFP nanobody conjugated Magnetic Beads	AT1773
RFP-Catch-Mag™ Anti-RFP tag nanobody conjugated Magnetic Beads	AT1774
mCherry-Catch-Mag™ Anti-mCherry tag nanobody conjugated Magnetic Beads	AT1775

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