PRODUCT DATA SHEET

FLAG-Catch-Aga™ Anti-FLAG Alpaca nanobody conjugated Agarose Beads

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

Catalog Number: AT1762

Size: 500 µL (250 µL settled beads), 1 mL (0.5 mL settled beads)

Store at: 4°C, **do not freeze**.

Background

Anti-FLAG agarose beads is an alpaca single domain antibody that is covalently attached to agarose 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-Aga™ Anti-FLAG tag nanobody (from alpaca VHH, Single Domain antibody) conjugated Agarose Beads
Form of product:	Agarose beads Conjugated anti-FLAG Nanobody, the product is supplied as a 50% suspension in 10 mM phosphate buffered saline (PBS), pH 7.4, and 0.02% (w/v) sodium azide as preservative.
Bead size:	~ 90 µm (cross-linked 8% agarose beads)
Specificity:	This antibody detects FLAG(DYKDDDDK)-tagged proteins exogenously expressed in animal cells, E. coli, yeast, insect cell, plant etc.
Reactivity:	Grab transfected FLAG fusion proteins expressed in E. coli, yeast, insect cell, mammalian cell, or in vitro transcription/translation systems.
Tested applications:	Immunoprecipitation (IP), Co-Immunoprecipitation (Co-IP), Chromatin Immunoprecipitation (ChIP) RNA Binding Protein Immunoprecipitation (RIP) Enzyme assays Mass spectrometry Affinity purification In IP, 25 µL of beads suspension for ~500 µL of crude total cell lysate solution. Optimal dilutions/concentrations should be determined by the end user.
Binding capacity:	25 µg of FLAG tagged GFP protein can be precipitated with 25 µL of beads suspension.
Host:	Alpaca
Clonality:	Monoclonal nanobody
Isotype:	Conjugated antibody isotype, Alpaca single domain antibody (nanobody, VHH)
Immunogen:	Recombinant flag tagged protein.
Storage instruction:	Store at +4°C and **do not freeze**.
Expiration Date:	Expires one year from date of receipt when stored as instructed

Attention: Agarose 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

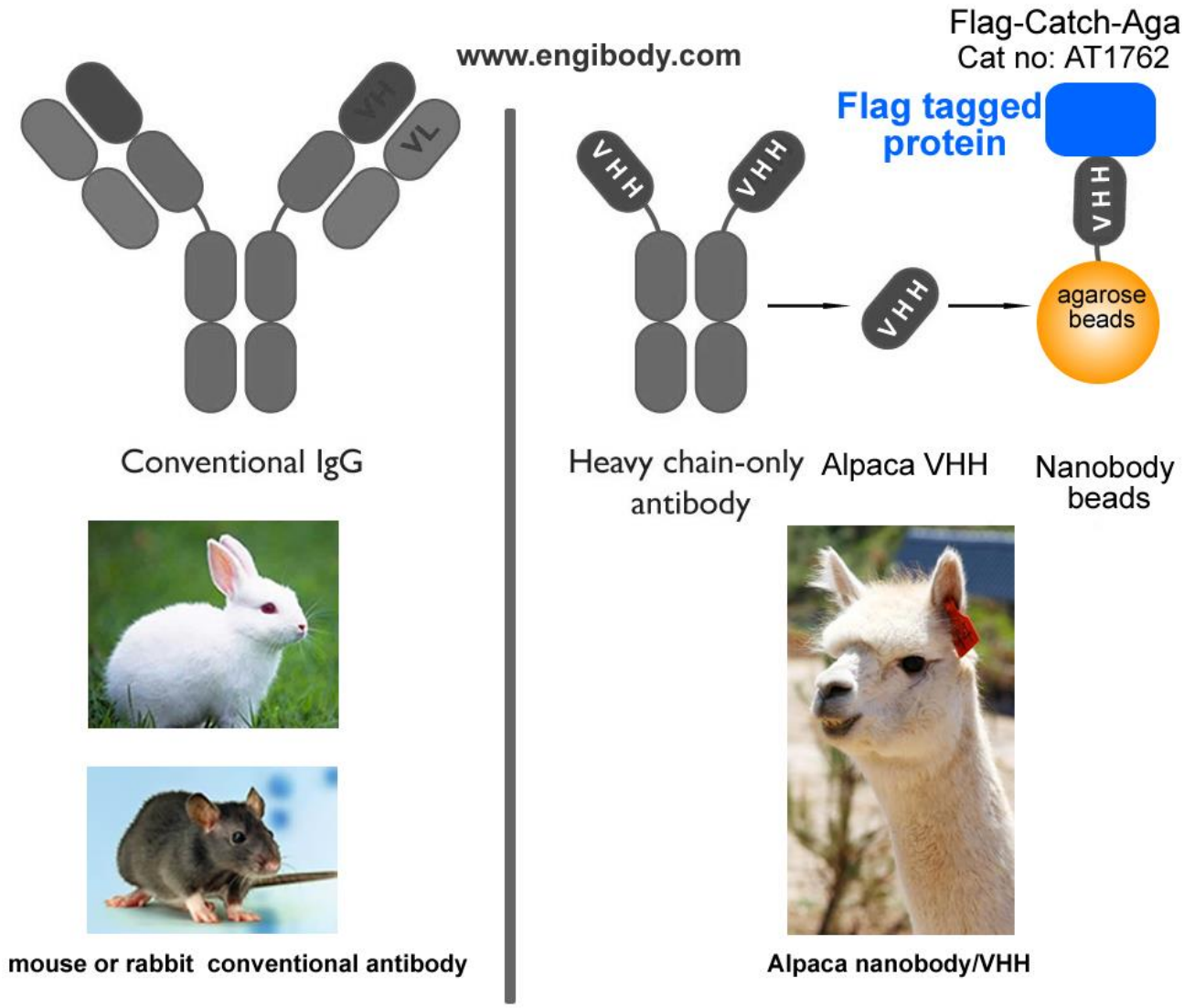
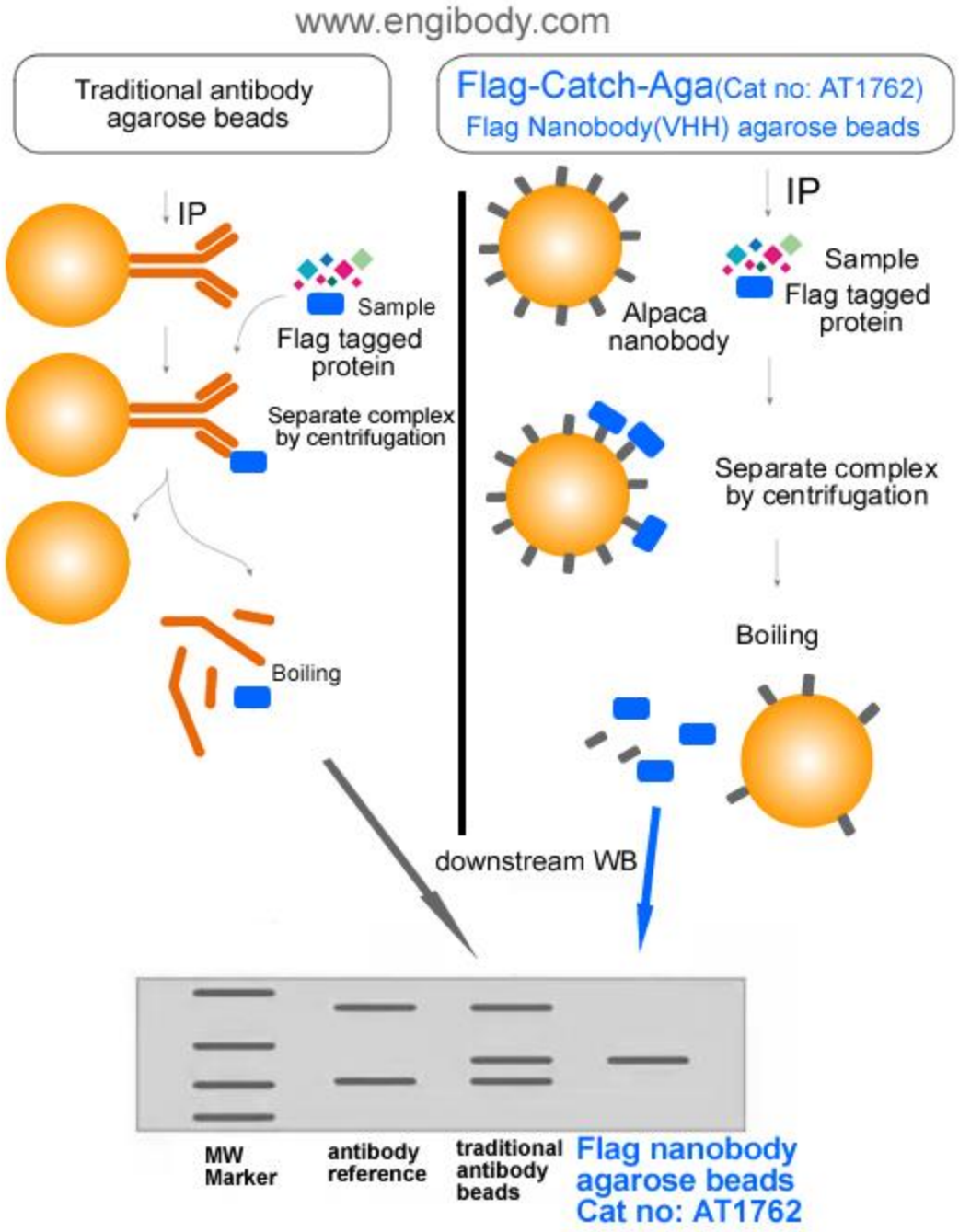
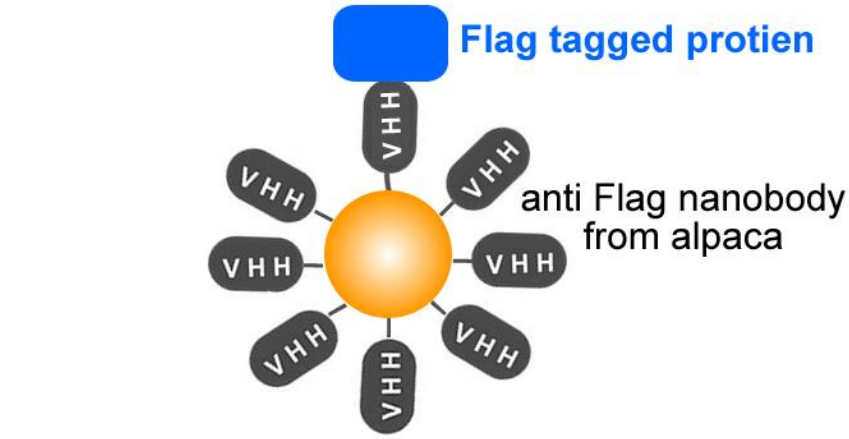


Figure 2. A schematic representation of benefits (no interfering of traditional antibody heavy and light chains)



Structure Image



Flag-Catch-Aga™
Anti-Flag nanobody conjugated Agarose Beads
Cat no: AT1762
www.engibody.com

Protocol for Immunoprecipitation (IP/Co-IP) 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. Harvest cells

1). For one immunoprecipitation reaction the use of ~10^6 - 10^7 mammalian cells (approx. one 10-cm dish) expressing a FLAG-tagged protein of interest is recommended. To harvest adherent cells, aspirate growth medium, add 1 mL ice-cold PBS to cells and scrape cells from dish. Transfer cells to a

pre-cooled tube, spin at 500 g for 3 min at +4°C and discard supernatant. Wash cell pellet twice with ice-cold PBS, gently resuspending the cells.

2. Lyse cells
1). Resuspend cell pellet in 500 µL ice-cold lysis buffer by pipetting.
Note: Add protease inhibitors and 1 mM PMSF into lysis buffer.

2). Place the tube on ice for 30 min with extensively pipetting every 10 min.

3). Centrifuge cell lysate at 14000x g for 10 min at +4°C. Transfer lysate(supernatant) to a pre-cooled tube. Discard pellet.
Note: At this point cell lysate may be put at -80°C for long-term storage.

3. Equilibrate beads
1). Resuspend the beads by inverting the product tube or gently pipetting up and down. Do not vortex the agarose beads,
2). Pipette 25 µL bead slurry into 500 µL ice-cold cell extract. Centrifuge at 2500x g for 2 min at +4°C. Discard supernatant and repeat wash twice.

4. Bind proteins
1). Add 25 µL equilibrated anti-FLAG agarose beads slurry (step 3) into 500 µL cell extract (step 2). Save 30 µL of cell extract for input of immunoblot analysis before IP. Incubation at a rotator for 1-3 hours at 4°C or overnight 4°C.

2). Centrifuge at 2500x g for 2 min at +4°C. Discard supernatant.

5. Wash beads
1). Resuspend protein-antibody-beads complex in 1mL ice-cold wash buffer. Centrifuge at 2500x g for 5 min at +4°C. Discard supernatant and repeat wash at least three times.

6. Elute 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 50-100 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. Centrifugate the beads complex at 2500x g for 5 min at 4°C. 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 4 °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 to dissociate immunocomplex from anti-FLAG agarose beads.

3. Anti-FLAG agarose beads immunocomplex can be centrifugated at 2500x g for 2 min at 4°C. 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. Centrifuge the resin for 30 seconds at 5,000–8,000g. 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
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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