V5-tag IP/Co-IP Kit (Nanobody Magnetic Beads)

V1506149

Storage -20°C, 4°C and room temperature (For detailed storage conditions, please refer to the Kit Components).

Shipping Shipped with ice packs. Upon receipt, please promptly store the product under the recommended storage conditions.

Product Introduction

V5 nanobody magnetic beads are conjugated with rigorously screened, optimized, and recombinantly expressed V5 nanobodies lacking light and heavy chains. They can be used to capture V5-tagged fusion proteins (GKPIPNPLLGLDST) and their interacting proteins from cell or tissue lysates of various biological sources, including mammals, plants, bacteria, yeast, and insects. This kit contains high-quality Anti-V5 magnetic beads and optimized, validated essential reagents for immunoprecipitation, making immunoprecipitation (IP, also known as pull-down) or co-immunoprecipitation (Co-IP) experiments simpler, more convenient, and efficient. It is widely applicable for immunoprecipitation, co-immunoprecipitation, or purification of fusion proteins or protein complexes carrying the V5 tag.

Tags such as V5-tag, His-tag, Myc-tag, Flag-tag, HA-tag, and GST-tag are among the most common tags used in expression vectors. Fusion expression with these tags enables convenient detection of target proteins and their interacting partners, as well as straightforward purification of the target proteins. This approach offers advantages such as simple operation, strong binding capability, and broad applicability. The storage temperature for components includes -20°C, 4°C and room temperature; for detailed storage information, please refer to Component Table 1.

Table1. Kit Components

V1506149	Components	20T	100T	Storage temperature	Quantity Per Test
V1506149A	Anti-V5 Nanobody Magnetic Beads	0.4 mL	2 mL	2-8°C. Do not freeze	20 μL
V1506149B	1×Lysis Buffer	20 mL	100 mL	2-8°C	150 μL per 250 μg sample
V1506149C	10×Wash Buffer	40 mL	200 mL	RT	0.5 mL
V1506149D	Protease Inhibitor Cocktail(100×)	0.2 mL	1 mL	-20°C	1.5 μL per 150 mL Lysis Buffer
V1506149E	2×SDS-PAGE Loading buffer	0.4 mL	2 mL	-20°C	20 μL per 30 μL beads

Note: For conventional immunoprecipitation experiments, use 20 μL of chromatography media

per 250 μg of sample.

Table 2. Performance of Anti-V5 Nanobody Magnetic Beads

Parameters	Specifications
Magnetic Bead Diameter	2 μm
Binding Capacity	⩾1.5 mg protein per mL of magnetic beads
Storage Buffer	20 mM PBS, 5% BSA
Storage Conditions	Store at 4°C. Do not freeze
Shelf Life	1 year

Procedure (Unless otherwise specified, all procedures should be performed at 4°C)

1 Reagent Preparation

Additional Required Materials:

(1) Reagents to be prepared by the user:

a) Primary Antibody: V5 tag antibody.

b) Secondary Antibodies: Goat Anti-Mouse IgG H&L (HRP), Goat Anti-Rabbit IgG H&L (HRP).

c) Other Reagents: TBST, Electrophoresis Buffer, Transfer Buffer, Reducing SDS-PAGE Gel.

(2) Required Equipment:

Electrophoresis Apparatus, Transfer Apparatus, Imaging System

The above reagents, if required, can be ordered from Aladdin: V5 tag Mouse mAb (Ab133823), Recombinant V5 tag Antibody (Ab086830), Goat Anti-Rabbit IgG H&L (HRP) (Ab176443), Goat Anti-Mouse IgG H&L (HRP) (Ab179001) and Goat Anti-Rabbit IgG H&L (HRP)Ab170144.

2 Solution Preparation

You may use the buffers provided in the kit, or prepare different buffer systems according to actual needs. It is recommended to filter all buffers through a 0.22 μm or 0.45 μm membrane filter before use. Buffers should be stored at 4°C. If any reagent appears cloudy, discard it immediately.

(1) Prepare an appropriate amount of inhibitor-containing lysis buffer based on the proportion of using 100-200 μL of inhibitor-containing lysis buffer for lysis and 300-600 μL of inhibitor-containing lysis buffer for washing per 0.5-1 million cells. Mix Lysis Buffer and Protease Inhibitor Cocktail (100×) at a ratio of 100:1. For example, add 10 μL of Protease Inhibitor Cocktail(100×) to 1 ml of Lysis Buffer to obtain 1 ml of inhibitor-containing lysis buffer (Lysis Buffer with Protease Inhibitor Cocktail). The prepared inhibitor-containing lysis buffer should be placed on ice or at 4°C.

Note: The inhibitor-containing lysis buffer should be prepared fresh before use and should not be frozen and stored for later applications.

(2) reparation of 10×Wash Buffer: Dilute the 10×wash buffer with deionized water at a 9:1 ratio. For example, add 9 mL of deionized water to 1 mL of 10×wash buffer, and mix well to obtain the 1×wash buffer.

(3) Beads Washing: Gently resuspend the Anti-V5 Magnetic Beads to form a homogeneous gel suspension. Typically, use 20 μL of the well-mixed gel suspension per 250 μg (the following immunoprecipitation steps are described based on adding 20 μL of gel suspension per sample). Transfer an appropriate amount of Anti-V5 Magnetic Beads into a clean centrifuge tube, and add 1×wash buffer to a final volume of approximately 0.5 mL. Allow to stand on the magnetic rack for 1 minute and discard the supernatant. Repeat the above steps twice.

Note: Using wide-bore tips (e.g., by cutting off the tip end with scissors) may facilitate pipetting of the gel suspension.

3 Preparation of Test Samples (**Note**: Perform all sample lysis steps at 4°C or on ice)

(1) For the preparation of serum samples:

If the target protein is abundant, dilute the serum sample with Lysis Buffer to a final target protein concentration of 50-150 μg/mL. Keep the diluted sample on ice for immediate use or store at -20°C for long-term preservation.

(2) For Adherent Cell Lysis and Preparation:

Aspirate the culture medium and wash the cells twice with PBS. Remove all residual liquid completely. Add 100-200 μL of Lysis Buffer with inhibitors per 0.5-1 million cells (equivalent to one well of a 6-well plate). Pipette gently to ensure thorough contact between the lysis buffer and cells. Animal cells are typically lysed within 1–2 seconds of contact with the buffer. For plant cells, lyse on ice for 2-10 minutes. After complete lysis, use a cell scraper to detach the cells and transfer the lysate to a 1.5 mL microcentrifuge tube. Centrifuge at 10,000-14,000×g for 3-5 minutes at 4°C. Collect the supernatant for protein concentration determination before proceeding to subsequent immunoprecipitation or co-immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, may be present after lysis and will form a pellet upon centrifugation.

(3) For Suspension Cell Lysis and Preparation:

Collect cells by centrifugation at 250-1,000×g for 5 minutes at room temperature. Wash the pellet twice with PBS and remove all residual liquid completely. Gently vortex or tap the tube to disperse the cells. Add 100-200 μL of Lysis Buffer with inhibitors per 0.5-1 million cells. Mix and incubate on ice for 5-20 minutes (mix several times during incubation). Tap the tube or pipette gently to ensure complete cell lysis; no significant cell pellet should remain after thorough lysis. If processing number of cells, aliquot them into tubes containing 0.5-1 million cells per tube before lysis. Large cell clumps are more difficult to lyse completely, whereas smaller numbers of cells allow better contact with the lysis buffer and lyse more efficiently. After complete lysis, centrifuge at 10,000-14,000×g for 3-5 minutes at 4°C. Collect the supernatant for protein concentration determination before subsequent immunoprecipitation or co-immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, may be present after lysis and will form a pellet upon centrifugation.

(4) For Bacterial or Yeast Sample Lysis and Preparation:

For 1 mL of bacterial or yeast culture, centrifuge to pellet the cells and remove the supernatant. Wash the pellet twice with PBS and remove all residual liquid completely. Gently vortex or tap the tube to resuspend and disperse the bacterial or yeast cells. Add 100-200 µL of Lysis Buffer with inhibitors. Gently vortex or tap the tube to mix, and lyse on ice for 2-10 minutes. For improved lysis efficiency, bacteria and yeast can be pretreated with lysozyme and lyticase, respectively, before adding the Lysis Buffer with inhibitors. After complete lysis, centrifuge at 10,000-14,000×g for 3-5 minutes at 4°C. Collect the supernatant for protein concentration determination before subsequent immunoprecipitation or co-immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, is likely present after lysis and will form a pellet upon centrifugation.

(5) For Tissue Sample Lysis and Preparation:

Mince the tissue into small fragments. Add approximately 100-200 µL of Lysis Buffer per 20 mg of tissue. Homogenize the mixture using a glass homogenizer or other suitable homogenization device. Thorough homogenization ensures complete tissue lysis. After lysis, centrifuge at 12,000×g for 5 minutes at 4°C. Collect the supernatant for protein concentration determination before subsequent immunoprecipitation or co-immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, is likely present after lysis and will form a pellet upon centrifugation.

4 Immunoprecipitation (IP)

(1) Beads Addition and Incubation

Add magnetic beads to the protein sample at a ratio of 20 µL bead suspension per 250 µg of protein. Incubate the mixture on a rocking platform or rotary mixer for 2 hours at room temperature or overnight at 4°C.

(2) Separation Using a Magnetic Rack

After incubation, place the tube on a magnetic rack and let it stand for 1 minute. Transfer the supernatant to a new microcentrifuge tube and save it for potential future analysis.

(3) Washing

Add 0.5 mL of Wash Buffer and resuspend the beads by gently pipetting up and down. Place the tube on the magnetic rack and let it stand for 1 minute. Discard the supernatant. Repeat the washing process three times using Wash Buffer. The protein-beads complexes are now obtained.

Note: The completeness of washing can also be monitored by measuring the OD_{280} of the wash solution. If the OD_{280} reading is greater than 0.05, increase the number of washes appropriately.

5. Protein Elution

Elution with SDS-PAGE Loading Buffer: For every 20 µL of original bead volume, add 30 µL of wash buffer to resuspend the beads, followed by 30 µL of 2×SDS Loading Buffer. Mix gently by flicking the tube, then heat at 95-100°C for 5-10 minutes. Centrifuge at 5000×g for 1 minute. Collect the supernatant for SDS-PAGE electrophoresis or Western Blot analysis.

Note 1: Since Anti-V5 nanobody magnetic beads are free from antibody heavy/light chain contamination, the denaturing elution method is preferred for higher elution efficiency.

Note 2: The recommended loading volume for WB is 20 μL or less. The remaining sample can be stored at -20°C.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
No Protein in Elution Fraction	Protein may be in inclusion bodies, not in the supernatant.	Check if the supernatant contains the target protein by analyzing the lysate via electrophoresis. Proteins in inclusion bodies require purification methods specific for inclusion bodies.
	Expression level is too low	Optimize expression conditions
	V5 tag is expressed but inactive	Optimize expression conditions
Non-specific bands are prominent	Insufficient washing during western blot	Increase the number of wash steps
Target Protein Not Captured	Insufficient amount of target protein in the sample	Verify protein expression or lysis efficiency by SDS-PAGE or Western Blot. Increase the amount of target protein to the recommended level
	Protein-protein interaction is too weak or non-existent	Optimize the Lysis/Wash Buffer; Add co-factors required for the protein interaction.
	Protein degradation	Add protease inhibitors; For temperature-sensitive proteins, perform all operations at 4°C or on ice.

Important Notes

1. This product is for research use only and restricted to scientific research by qualified professionals.
2. Please read this instruction manual carefully before performing protein–protein interaction assays.
3. Unless otherwise stated, all procedures are recommended to be performed at 4°C to minimize protein degradation. If cell lysis is incomplete, sonication may be applied after treatment with lysis buffer.
4. Anti-V5 Nanobody magnetic beads should be stored in storage solution to prevent drying. Mix thoroughly by gentle inversion several times before use. Vigorous vortexing is not recommended to avoid antibody denaturation.
5. Boiled beads lose their binding capacity and should not be reused.
6. Reagent volumes may be adjusted according to actual experimental conditions. Preliminary experiments are recommended to verify immunoprecipitation or optimize lysis conditions.
7. Please observe safety precautions and comply with standard laboratory reagent handling protocols.

8. The lysis buffer included in this kit already contains protease inhibitors. Other appropriate inhibitor cocktails may be used for specific requirements.
9. Protein samples should be purified promptly after collection and kept at 4°C or on ice to reduce protein degradation or denaturation.
10. Positive and negative controls are recommended for immunoprecipitation or purification experiments.