Anti-DYKDDDDK Affinity Gel

Product Packaging:：

Product number	Product name	Product form	Package size （V/V, 1/2）
HAK21024-500 ul	Anti-DYKDDDDK Affinity Gel	50 % suspension	500 uL
HAK21024-2 mL	Anti-DYKDDDDK Affinity Gel	50 % suspension	2 mL
HAK21024-10 mL	Anti-DYKDDDDK Affinity Gel	50 % suspension	10 mL

Product Description:

The affinity gel is designed for immunoprecipitation and purification of proteins for various research purposes. This product is covalently conjugated with anti-DYKDDDDK [A2-A4] with a diameter of 46-165 μm, specifically designed to recognize proteins tagged with a Flag peptide sequence at either the methionine-modified N-terminus or C-terminus. This product is particularly useful for capturing and purifying Flag-fused recombinant proteins of mammalian, bacterial or plant origin.

Product performance:

Form: The product is supplied as 50 % slurry in PBS, 0.05 % ProClin™300，pH 7.4

Binding capacity: ≥0.8 mg C-terminal fusion Flag tag protein (~70kDa)/1mL settled affinity gel

Storage：

Store at 2-8°C for 2 years. DO NOT freeze or centrifuge affinity gel

Important Licensing Information：

For laboratory or further manufacturing use only.

Instructions for use:

1. Sample preparation

In order to achieve optimal experimental outcomes, careful sample preparation is essential. Follow these guidelines to ensure the quality of your protein lysate:

1.1 Controlling pH and Salt Concentration:

Adjust the pH of the protein lysate to a range of 6 to 8. This pH range enhances binding efficiency during subsequent steps. Maintain a minimum concentration of 0.15M of NaCl or KCl in the protein lysate. This salt concentration promotes favorable protein interactions.

1.2 Centrifugation for Debris Removal:

Centrifuge the protein lysate at a speed of 10,000 to 20,000 × g for 15 minutes. This centrifugation step effectively removes cellular debris and unwanted particles that could potentially hinder protein binding.For better results, protein lysate can also be filtered through a 0.45 μm or 0.22 μm filter.

2. Immunoprecipitation (IP)

This procedure is recommended for purifying small amounts of Flag fusion protein. It is recommended to use 20-30 µL of affinity gel suspension per reaction (1x10^6 cells or 500 µL lysate) for IP.

1.Suspend the gel in the bottle thoroughly and transfer the required amount of affinity gel into an empty tube.

2. Use TBS buffer to wash affinity gel with 10 volumes of the original gel suspension, Collect the affinity gel with a centrifugation of 6,000 × g for 30 seconds, and remove the supernatant. Repeat 3 times.

3. Add 500 µL of cell lysate sample to the prepared affinity gel (the volume of the lysate can be adjusted according to the abundance of the target protein in the sample). For the positive control, add 100 uL TBS and ~200ng Flag fusion protein to the pre-washed affinity gel. For the negative control, add only 500 uL of lysis buffer without protein.

4. Mix by rotation on a rotator for at least 1 hour at room temperature or overnight at 4°C.

5. Collect the affinity gel with a centrifugation of 6,000 × g for 30 seconds. Remove and discard the supernatant.

6. Use TBS buffer to wash gels with 10 volumes of the original affinity gel suspension. Repeat 3 times.

7. Elution: According to the requirements of subsequent experiments, one of the following three methods can be selected for elution.

7.1 Elution with acid elution buffer

This is a non-denaturation method, which is fast and efficient. Protein can retained its original biological activity after elution, which was convenient for subsequent analysis and detection.

7.1.1 Preparation of solution: acid eluent (0.1M glycine, 0.2M arginine, pH2.7), neutralizing solution (1M Tris-HCl, pH9.0).

7.1.2 Add 100 uL acid elution to 10ul affinity gel suspension and mix well, then place on a rotating mixer. Incubate the samples for 5 minutes at room temperature. (Note: Do not leave the gels in this buffer more than 15 minutes.)

7.1.3 Transfer the supernatants to fresh tubes, and immediately add 10ul neutralizing solution.

7.1.4 Repeat steps 7.1.2-7.1.3 in order to improve elution efficiency, pooling same eluates in tube.

7.1.5 The eluted and neutralized target protein for immediate use, store the eluates at 2-8 °C. Store at –20 °C for long term storage.

Note: 1) Although the acidic elution method is efficient, it may still be lower than the SDS-PAGE sample buffer elution method. 2) Since the difference of the target protein may have a certain influence on the elution efficiency of the acidic elution method, if the elution efficiency is relatively high, the pH of the acid eluent can be adjusted between 2.5-3.1, and the pH value or amount of the corresponding neutralizing solution should also be adjusted to a certain extent.

7.2 Elution with SDS-PAGE Loading Buffer

This is a denaturation method. The protein samples obtained by this method are suitable for SDS-PAGE electrophoresis or Western Blot detection.

7.2.1 Dilute 5X SDS-PAGE loading buffer to 1X with PBS.

7.2.2 Add 50 µL 1X SDS sample loading buffer to 10ul affinity gel suspension and Boil the sample for 10 minutes at 95°C.

7.2.3 Transfer the supernatants to fresh tube. The sample are ready for loading on SDS-PAGE and immunoblotting.

Notes: Do not re-use affinity gel after treatment with SDS-PAGE buffer.

7.3 Elution with competitive elution buffer

This is a non-denaturation method, which is fast and efficient. Protein can retained its original biological activity after elution, which was convenient for subsequent analysis and detection.

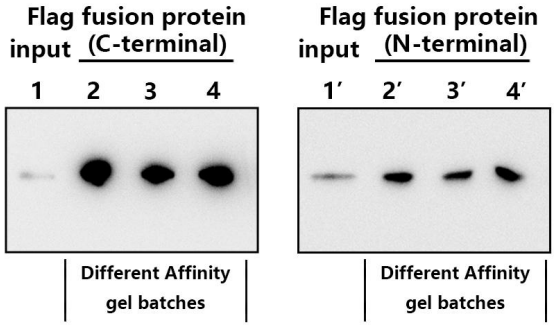
7.3.1 Preparation of 3X HA peptide elution at 300 µg/ml in TBS with 1 %Triton and 0.1 %SDS.

7.3.2 Add 100 uL 3X HA peptide elution to 10ul affinity gel suspension and mix well, then place on a rotating mixer. Incubate the samples for 30-60 minutes at room temperature.

7.3.3 Collect the affinity gel with a centrifugation of 6,000 × g for 30 seconds and then remove the supernatant to a clean tube.The supernatant is the target Flag-tagged protein.

7.3.4 Store the eluates at 4 °C for immediate use. Store at –20 °C or –80 °C for long term storage.

4. Validation results



Immunoprecipitation

anti-DYKDDDDK [A2-A4] Affinity Gel: Purification of two different flag-tagged fusion protein from HeLa cells transfected with Flag-fusion protein expression vector. 10ul anti-DYKDDDDK [A2-A4] Affinity Gel was used for IP per lane.

Lane 1, 2, 3, 4: HeLa transfected with Flag-fusion protein expression vector containing an C-terminal Flag tag, whole cell lysate

Lane 1', 2', 3', 4': HeLa transfected with Flag-fusion protein expression vector containing an N-terminal Flag tag, whole cell lysate