



## Anti-DYKDDDDK Agarose Gel, 30~100 $\mu$ m

### PRODUCT DATA SHEET

## Anti-DYKDDDDK Agarose Gel, 30~100 $\mu$ m

**Cat No: AG-Anti-DYKDDDDK-3000**

#### Description

Anti-DYKDDDDK agarose gel is a homemade agarose gel with high quality mouse anti-FLAG monoclonal antibody as ligand. It has high physical and chemical stability, and the ligand is not easy to fall off. It has a long life and is easy to use. This product is widely used in the immunoprecipitation (IP) or immunoco-precipitation (Co-IP) of DYKDDDDK labeled fusion protein in cell lysate, cell secreted supernatant, serum, ascites and other samples, and can also be used for the purification of DYKDDDDK labeled fusion protein.

For custom sizes, formulations or bulk quantities please contact our customer service department.

**Website:** [www.abvigen.com](http://www.abvigen.com) **Phone:** +1 929-202-3014 **Email:** [info@abvigenus.com](mailto:info@abvigenus.com)

#### Characteristics

Matrix	4% highly cross-linked agarose gel
Ligand	Murine monoclonal antibody against DYKDDDDK
Particle size	30~100 $\mu$ m
Concentration	50% (v/v)
Binding ability	$\geq 1.0$ mg DYKDDDDK tag protein/mL gel
Scope of application	IP, Co-IP, DYKDDDDK label protein purification
Shelf life	Stable storage at 2 ~ 8°C for 2 years

#### Operation process

**Note: Prepare your own buffer or purchase our IP kit directly.**

#### Adherent cell sample:

1. Remove the medium and wash the cells twice with PBS.
2. Cells were collected into 1.5 mL EP tube and IP Lysis/Wash Buffer was added proportioned, and add corresponding inhibitor such as PMSF. After mixing, the cells were placed on ice for 5 ~ 20 min (mixed several times).

3. At 4°C, 12000 ~ 16000 xg, centrifuge for 10 min to collect the supernatant and place it on ice for subsequent experiments (or place it at -80°C for long-term storage).

**Petri dish IP Lysis/Wash Buffer Recommended volume:**

Petri dish size/surface area	Volume of IP Lysis/Wash Buffer
100 mm x 100 mm	500 ~ 1000 $\mu$ L
100 mm x 60 mm	100 ~ 300 $\mu$ L
6-well plate	100 ~ 200 $\mu$ L

**Suspended cell sample:**

1. Cells were collected at 4°C, 500 ~ 1000 xg, 10 min, and the supernatant was discarded.
2. Wash the cells with PBS once, that is, suspend the cell mass with PBS at 4°C, 500 ~ 1000 xg, 5 min, collect the cells, discard the supernatant.
3. Resuspend cells with pre-cooled IP Lysis/Wash Buffer. The 500  $\mu$ L IP Lysis/Wash Buffer was used for every 50 mg cells. At the same time, add the corresponding inhibitors such as PMSF, mix well and put on the ice for 5 ~ 20 min (mix several times during the period).
4. At 4°C, 12000 ~ 16000 xg, centrifuge for 10 min to collect the supernatant and place it on ice for subsequent experiments (or place it at -80°C for long-term storage).

**Serum sample:**

It is recommended to dilute a serum sample with IP Lysis/Wash Buffer to a final concentration of target protein of 50 to 150  $\mu$ g/mL and place it on ice for future use (or place it at -20°C for long-term storage).

**Immunoprecipitation:**

**Note:** In order to ensure the uniform distribution of gel, mix the gel in the bottle by repeatedly reversing or slightly vortexing before use.

1. Add 20 to 50  $\mu$ L of Anti-DYKDDDDK agarose gel into 1.5 mL centrifuge tube.
2. Add 500  $\mu$ L pre-cooled PBS to the gel and mix gently.
3. Put the centrifuge tube into the centrifuge at 1000 rpm for 5 min to collect the gel to the bottom of the centrifuge tube and remove the supernatant.
4. Add 200 ~ 500  $\mu$ L IP Lysis/Wash Buffer to the centrifuge tube. Reverse the centrifugal tube several times or mix with a slight vortex for 1 min. Put the centrifuge tube into the centrifuge at 1000 rpm for 5 min to collect the gel to the bottom of the centrifuge tube and remove the supernatant.
5. The prepared protein sample containing DYKDDDDK label was added into the centrifuge tube with gel and incubated at room temperature for 1 ~ 2 h, or 4°C 2 ~ 4 h.



6. Centrifuge at 1000 rpm for 5 min to collect gel, remove unbound sample and store for analysis.
7. Add 1000  $\mu$ L IP Lysis/Wash Buffer to the centrifuge tube and gently mix for 5 ~ 10 min. Collect the gel and discard the supernatant. Repeat twice.
8. **Denaturing elution:** Add 80 ~ 100  $\mu$ L SDS-PAGE Sample Loading Buffer (1 $\times$ ) to the centrifuge tube and heat the sample in 100°C water bath or metal bath for 10 min. Gel is separated by centrifugation to retain the supernatant containing the target antigen.

**Note:** The following elution methods can also be used to maintain protein activity.

**Low pH Elution:** Add 100  $\mu$ L Elution Buffer to centrifuge tube. Keep mixed and incubate centrifuge tube at room temperature for 5 ~ 10 min. Gel is separated by centrifugation to retain the supernatant containing the target antigen. Low pH is neutralized by adding 20  $\mu$ L Neutralization buffers per 100  $\mu$ L of wash out solution.

## Notes

1. Before performing the experiment, please read this operation manual carefully.
2. The affinities between different types of antibodies and antigen Lysis are different in IP experiments. The binding of antibodies to antigens is also affected by IP Lysis/Wash Buffer, so **you can do your own experiments by optimizing the operation details or screening and formulating buffers.**
3. Agarose gel should be fully oscillated and uniform before use. Gel should be kept in storage solution to prevent drying.
4. This product is for scientific research only.

## Problem solving

### 1. No immunoprecipitation of antigen

- ① The sample contains too little antigen to be detected

**Recommendation:** To verify the expression and/or cleavage efficiency of proteins in the lysate by SDS-PAGE or protein immunoblotting; If necessary, increase the sample size

- ② The ingredients in IP Lysis/Wash Buffer interfere with the binding of antigens to antibodies

**Recommendation:** Immunoprecipitation and rinsing with other buffers (e.g., TBS containing 0.5% CHAPS).

### 2. Low protein intake

- ① The protein is degraded

**Recommendation:** Add protease inhibitor



② The amount of gel used is not enough

**Recommendation:** Increase the amount of gel used to capture immune complexes

③ The amount of target protein in the sample is not enough

**Recommendation:** Increase the amount of antigen sample

### **3. Multiple non-specific bands**

There are non-specific proteins that bind to the gel

**Recommendation:** Add 50 ~ 350 mM NaCl to IP Lysis/Wash Buffer. Increase elution intensity and frequency.

### **4. The gel sticks easily to the tube wall**

**Recommendation:** Use consumables with low adsorption rates for gel manipulation.

### **Ordering Information**

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