DDKTrap™ Anti-DDK Affinity Resin

1. Product Description

The DDK, DYKDDDDK, or FLAG® tag is a hydrophilic short peptide specially designed for protein purification and detection, consisting of eight amino acids (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys, abbreviated as DYKDDDDK), with a molecular weight of about 1kD. This tag is also called the DDK tag. The tag is often optimally designed and placed on the surface of a protein construct, making it convenient for purification and detection by being accessible to bind to the antibody-coated resin. OriGene DDKTrap™ Anti-DDK Affinity Resin uses anti-DDK antibodies as affinity ligands. It can be used to purify Flag-tagged fusion proteins expressed in prokaryotic, yeast, or mammalian cells in one step.

Characteristic	Performance
Matrix	4% agarose microspheres
Binding Ligand	OriGene's Anti-DDK Antibody, Clone 8G6
Target Protein Binding Capacity	>1mg DDK-tagged protein/ml resin
Resin Particle Size Range	45 – 165 µm
Maximum Column Pressure	0.1 MPa
Storage Buffer	50% glycerol, 1 x PBS, 0.02% NaN_3
Storage Temp. and Stability	Stable at -20°C for 1 year

2. Reagent Preparation

2.1 Sample Preparation

It is recommended to centrifuge crude lysate or use a 0.45µm membrane filtration to reduce impurities, improve protein purification efficiency, and prevent clogging.

2.2 Buffer Preparation

Acidic Elution Buffer: 0.1 M glycine HCl, pH 3.0

Neutralizing Buffer: 1 M Tris-HCl, pH 8.0

(Alternative Competitive Eluent: 50mM Tris, 0.15 M NaCl, 100-500 µg/ml flag peptide, pH7.4

Note: It is recommended to use purified water, which is filtered with a 0.45 µm filter membrane before use.

3. Protein Purification Protocol

3.1 Resin Equilibration

Resuspend the resin slurry with gentle rotation (2 ml slurry = 1 ml resin). Equilibrate the desired volume of resin with 5 bed volumes of the buffer compared to the crude protein sample, thus removing the resin storage buffer. Resin can be separated from the storage buffer and equilibrated with a new buffer on a disposable column or via brief centrifugation at 300g.

3.2 Resin Binding (2 Options)

a. Bulk Resin Binding: Add the equilibrated DDKTrap™ Anti-DDK Affinity Resin to the crude protein sample and incubate at room temperature for >30 min or 4°C for >1 hr. Gently rock or end-over-end rotate the sample for the duration of the resin binding incubation. Performing resin binding at 4°C overnight can boost protein binding further in many circumstances if this time scale is compatible with your protein of interest. Centrifuge the resin/sample mixture at 300g for 10 min to pellet the resin. Gently decant or pipette off the supernatant and load the pelleted resin onto a column.

b. Column Resin Binding: Add your crude protein sample to a column of equilibrated DDKTrap™ Anti-DDK Affinity Resin, making sure to collect the column flow through in a clean tube/vessel. Pass the flow through again through the column at least 3 times to increase the binding quantity.

3.3 Column Washing

Wash the protein-bound resin with 10-20 column volumes of wash buffer of your choice to remove non-specifically bound proteins and collect the washing solution. It is recommended that the wash buffer flow completely to the surface of the resin bed (without letting the resin dry) before adding each aliquot of wash buffer.

3.4 Protein Elution (3 Options)

a. Acidic Elution Method: Add at least 5 column volumes of Acidic Elution Buffer (0.1 M glycine HCl, pH 3.0) to elute all protein, and quickly balance the pH with a 1:20 volume of Neutralizing Buffer (1 M Tris-HCl, pH 8.0) to raise the pH to 7.4. To achieve the highest concentration possible, divide the 5 column volumes worth of Acidic Elution Buffer into smaller fractions to avoid over-diluting the final sample. We suggest fractions that are ½ of the resin bed volume.

Note: After acidic elution, the resin must be equilibrated with a neutral buffer if intended for re-use. Anti-DDK Affinity Resin should not be left in the eluent for more than 20 minutes.

b. Competitive Elution Method: Eluting with 5 column volumes of a competitive (50mM Tris, 0.15 M NaCl, 100-500 µg/ml FLAG peptide, pH 7.4) can also elute the protein from the resin. While no pH adjustment is needed, buffer exchanging or desalting the solution to remove the competitive peptide may be required, depending on your use case.

c. Denaturing Elution Method: Using denaturing conditions buffer conditions can also elute the DDK-tagged protein from the resin, however, it should be noted that reducing agents and more vigorous detergents such as SDS will separate the antibody heavy chains and light chains which are bound to the resin, meaning antibody anti-DDK protein will likely be present in your final sample.

3.5 Regeneration

Once finished eluting the protein, at least 3 column volumes of a neutral equilibration buffer of choice can be used to restore a neutral pH of the column so that the resin may be reused.

3.6 Storage

The restored resin can be stored for a short period of time in PBS containing 0.02% NaN_3 at 2-8°C.

4. Immunoprecipitation Protocol

4.1 Resin Preparation

Take 40 µl of DDKTrap™ Anti-DDK Affinity Resin (resin bed volume 20 µl), add to a 2 ml centrifuge tube, and centrifuge at 5,000 g for 1 min. Aspirate and discard the storage buffer. Add 0.5 ml of desired equilibration buffer (typically the buffer the immunoprecipitation sample is in), resuspend the pelleted resin, and then pellet again at 5,000 g for 1 min and discard the supernatant. Repeat this process of washing the resin with 0.5ml of equilibration buffer once more to complete the process.

4.2 Resin Binding

Add 200-1,000 µl of sample lysis solution to the equilibrated resin, mix evenly, place the centrifuge tube in an inversion mixer at room temperature, and gently rotate the centrifuge tube to promote full binding and adsorption of the sample to the resin for at least 1 hr. Centrifuge at 5,000 g for 1 min and remove the supernatant.

4.3 Washing

Add 0.5 ml washing solution (typically another buffer of your choice compatible with your sample of interest) to the resin, mix gently, and centrifuge at 5,000 g for 1 min, aspirate, and remove the supernatant. Repeat 3 more times.

4.4 Elution

One of the 3 elution methods described in section 3 (Protein Purification Protocol) can be used to elute the protein from the resin for further analysis.

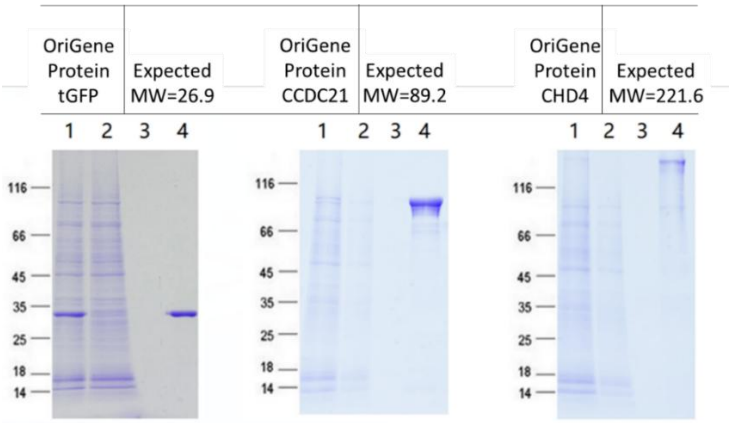
5. Reagent Compatibility

Reagent Name	Maximum Tolerated Concentration	Note
β -Mercaptoethanol	10 mM	May detach the antibody from the resin, and the resin will not be able to be reused after elution
DTT	80 mM	
SDS	-	
EDTA	5 mM	High concentrations will reduce the efficacy of resin if it is reused
Tween- 20	5%	Higher concentrations will affect the binding efficiency of the target protein.
Triton X- 100	5%	
NP- 40	4%	
Guanidine hydrochloride	0.3 M	Denaturing agents may hamper the resin's ability to be reused
Urea	1.5 M	
Glycerol	20%	High concentrations may reduce binding
NaCl	1 M	Can help reduce non-specific binding

6. Troubleshooting

Troubleshooting Issue	Potential Cause	Recommended Solution
Target protein is present in the flow through	Resin is overloaded	Reduce the sample volume or increase the resin volume
	Binding time is too short	Prolong the incubation time
	Tag not exposed on the surface of the protein	A low concentration of denaturant may expose the tag
	Buffer reagent incompatibility	Dialyze the sample into a compatible buffer
There is no target protein in the eluted fraction	Target protein is unstable	Use fresh samples and use 4°C conditions
	Expression of the target protein is too low	Optimize expression conditions. Check the sample via western blot before purifying. Reduce NaCl concentration of buffers to increase binding.
Final eluted fraction of protein is impure	Non-specific adsorption of protein to the resin	Reduce the quantity of sample loaded onto the resin and/or increase the [NaCl] of the lysis buffer
	Insufficient washing	Increase the volume of buffer used to wash the resin after bead binding. Consider increasing the [NaCl]

6. Purification Examples



Demonstration of the high level of protein purity achieved through the purification process of 3 DDK-tagged proteins of varying molecular weight.

Lane 1: Crude cell lysate containing the DDK-tagged protein

Lane 2: Sample flow through after the sample was incubated with the resin

Lane 3: Flow through from 20 column volumes of wash buffer (25mM Tris HCl pH 7.4, 500mM NaCl)

Lane 4: Pure eluted protein of interest

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