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DATASHEET

Anti-DYKDDDDK Affinity Resin Easy

Cat. No.: L00907

Contents

The operator must carefully read the datasheet before using this product.
This product is for research use only, not for clinical diagnosis.

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1. Description

The DYKDDDDK tag is an octapeptide tag that can be added to proteins through the recombinant DNA technology. It can be fused to the N-terminus or C-terminus of the target protein, facilitating the detection and purification of recombinant proteins. GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No. L00907) features high binding capacity, specificity, sensitivity, stability, and reusability, making it particularly useful for the affinity purification and immunoprecipitation of DYKDDDDK-tagged fusion proteins expressed in common protein expression systems such as bacteria, yeast, and mammalian cells.

Table 1. Characteristics of Anti-DYKDDDDK Affinity Resin Easy

Features	Specifications
Particle concentration	50% slurry in PBS, 0.02% Sodium azide
Application	Purification; Immunoprecipitation
Matrix	4% cross-linked agarose
Binding capability	~1 mg DYKDDDDK-tagged protein (~55 kDa) per ml settled resin
Particle size	45-165 µm
Ligand	Monoclonal antibody against DYKDDDDK tag
Storage	Store at 2°C~8°C. **Do not store at or below 0°C to avoid freezing the resin.**

2. Instructions for Use

Considering the complexity of tested samples and the diversity of experimental conditions, the following instructions can be used as a reference. It is recommended to perform a pre-experiment to achieve the optimized experiment conditions.

2.1. Purification of DYKDDDDK-tagged protein

2.1.1. Equipment and reagents needed but not supplied

- Micropipettes
- Centrifuge tubes
- Rotator
- Empty chromatography columns
- Protease inhibitor reagents
- Buffers, see Table 2

Table 2. Buffers required for the purification of DYKDDDDK-tagged protein

Purpose	Buffer	Recommend Formulation
Cell lysis	Tris-buffered saline (TBS)	50 mM Tris-HCl, 150 mM NaCl, pH 7.4
	Home-made or purchased commercial product	
Equilibrium	Tris-buffered saline (TBS)	50 mM Tris-HCl, 150 mM NaCl, pH 7.4
Washing		
Elution	Alkaline eluent	100 mM Tris, pH 12.0
	Competitive peptide eluent	0.1-0.4 mg DYKDDDDK peptide/ml in TBS or 0.4-1.0 mg MDYKDHDGDYKDHDIDYKDDDDK peptide/ml in TBS
Regeneration	Alkaline eluent	100 mM Tris, pH 12.0
Storage	Tris-buffered saline with ProClin 300 (TBSP)	50 mM Tris-HCl, 150 mM NaCl, pH 7.4, with 0.03% ProClin 300

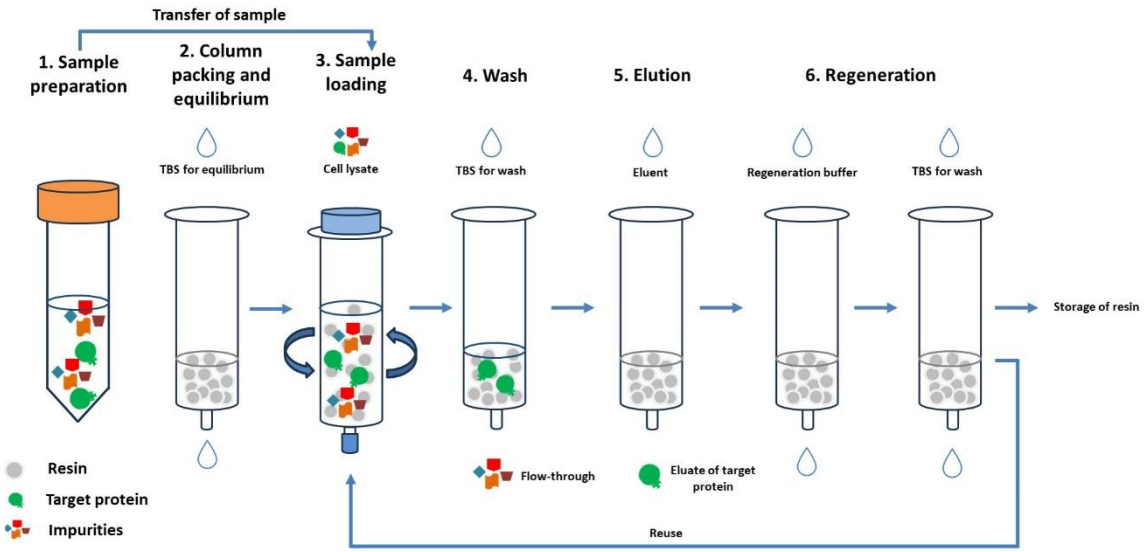


Figure 1. Flow chart of purification of DYKDDDDK-tagged protein

2.1.2. Sample preparation

Before loading samples to the resin, please refer to the following steps for sample preparation. If an in-house designed process is used, please make sure that the samples are centrifuged or filtered to prevent the column clogging during the purification process.

2.1.2.1. Preparation of intracellularly expressed protein samples

If the proteins are expressed intracellularly, a cell lysis process is needed. Add the cell lysis buffer to the cells, and then lyse the cells by sonicating or other proper methods. Centrifuge the mixture and collect the supernatant to a new centrifuge tube. Filter the supernatant with a 0.22 μm filter and save the filtrate for further experiment. If a commercial cell lysis product is used, please refer to the supplier's manual for instructions.

2.1.2.2. Preparation of extracellularly expressed protein samples

If the proteins are expressed extracellularly, centrifugation of the sample is needed, the collected supernatant needs to be filtered with a 0.22 μm filter and save the filtrate for further use.

Notes:

1) During the cell lysis process, if the viscosity of the sample is high, it is recommended to add an appropriate amount of endonucleases (GenScript, Cat. No. Z03695 Benz-Neburase™, tag-free), which can reduce the sample viscosity caused by the release of chromosomal DNA or RNA from host cells.
2) During the cell lysis process, certain chemical reagents may be used depending on the customers' needs varying with protein characteristics and expression system. In this case, please refer to "*Table 4. Reagent Compatibility*" in Section 3 for commonly tolerated concentrations of common chemical reagents for this product.
3) During the cell lysis process, if the expression level of the target DYKDDDDK-tagged fusion protein is low, methods can be used to concentrate the target protein: for the intracellularly expressed proteins, a reduced volume of TBS or cell lysis buffer can be used; for extracellularly expressed proteins, please concentrate the supernatant of lysis by ultrafiltration or other appropriate methods.

2.1.3. Column packing and equilibrium

Step 1. Preparation of the empty chromatography columns. Choose an empty chromatography column with a proper size based on the sample volume. Set the column onto a stand vertically, keeping both sides of column open, and rinse it with TBS once.
Step 2. Preparation of resin slurry. Use a rotator to mix the sealed resin for 5 minutes, until the resin forms a homogeneous suspension.
Step 3. Packing the resin. Use a pipette tip with a diameter greater than 1 mm (the tip can be cut by approximately 1-2 mm off with a pair of clean scissors) to aspirate the homogeneous resin suspension from 2.1.3 step 2 into the empty column from 2.1.3. step 1. Record the volume of settled resin as the bed volume of column. **Note:** The amount of resin used for packing should be estimated depending on the amount of target protein and the binding capacity of the resin. For example, generally, 0.1-1 ml of resin can be used for 1 ml~50 ml of sample.
Step 4. Equilibrium. Add 10 bed volumes of TBS into the packed resin.
Step 5. Allow the TBS to flow through the resin and drain through the bottom of the column. When the liquid level reaches the top of the resin, plug the bottom of the column with a stopper to keep the resin wet.
Note: To prevent a decreased purification efficiency due to the dry resin, it is essential to maintain the liquid level higher than the top of resin to keep the resin wet. If the resin becomes dry, add 2-3 bed volumes of TBS immediately and mix the resin and buffer thoroughly with either a pipette or a syringe. Let the resin settle down, then follow "*2.1.3 Step 5*" to get the column ready for the further use.

2.1.4. Sample loading

Based on the sample volume, methods in "*2.1.4.1 Incubation method*" or "*2.1.4.2 Flow through under gravity*" can be chosen to load the sample to the resin from "*2.1.3 Column packing and equilibrium*".

2.1.4.1. Incubation method

For a sample volume of more than 1 ml, an incubation process can be used for sample loading with the following instructions.

Step 1. Transfer of the sample. Check the column from "*2.1.3 Step 5*", please make sure the resin is wet. Set the column on a stand vertically, then aspirate the prepared sample from "*2.1.2 Sample preparation*" and transfer it to the column through the top of the column. Plug the top of the column with another stopper. If the sample volume exceeds the bed volume of resin, re-suspend the sample and resin in the column and transfer the suspension to a suitable container, add the remaining sample to the container for further incubation.
Step 2. Binding of the target proteins. Place the column or the suitable container on a rotator. Incubate it for 30 minutes at room temperature or overnight at 2°C~8°C with continuous rotation to ensure a sufficient binding between the sample and resin.
Step 3. Collection of the flow-through[1] solution. After incubation, set the column on a stand vertically and place an uncapped clean centrifuge tube under it. Open the top of the column and then the bottom of it to allow the solution in the column drain into the centrifuge tube. The collected solution can be labeled as flow-through and saved for further use. If the sample and resin are incubated in a container, transfer the suspension into a column and then collect the flow-through solution for further use. **Keep the resin wet throughout the entire process.**

*1. The collected flow-through solution during the process can be used for the checking of the process. Test the flow-through with western blotting or SDS-PAGE to determine whether the target proteins are in it. If a large quantity of target protein is found in the flow-through, please refer to the **Trouble Shooting Table** in section 5 for further optimization of the purification or immunoprecipitation process.*

2.1.4.2. Flow through under gravity

For a sample volume between 50 ml~100 ml, it is recommended to directly flow the sample through the column under gravity with the following instructions.

Step 1. Loading of the sample. Check the column from "*2.1.3 Step 5*", please make sure the resin is wet. Place an uncapped clean centrifuge tube under the column, and open the bottom of it. Then aspirate the prepared sample from "*2.1.2 Sample preparation*" and transfer it to the column through the top of it.
Step 2. Collection of the flow-through solution. After the sample loading, allow the sample solution to slowly flow through the column under gravity into the clean centrifuge tube for further use.
Note: Depending on the properties of the target protein and the flow rate during sample loading, there may be insufficient binding between the protein and resin for the first loading. In this case, it is recommended to perform repeated "*2.1.4.2 Flow through under gravity*" process for a couple of times to achieve an enhanced binding efficiency. **Keep the resin wet throughout the entire process.**

2.1.5. Wash away of the impurities

After the collection of flow-through, add 20 bed volumes of TBS to the resin. Let the TBS buffer drain through the bottom of the column. Plug the bottom of the column with a stopper when the TBS buffer level reaches the top of the resin. **Keep the resin wet throughout the entire process.**

2.1.6. Elution

Depending on the variations of the target protein and downstream applications, different elution methods can be selected. The following elution instructions are provided for reference.

2.1.6.1. Alkaline elution

Step 1. Incubation. Add 10 bed volumes of alkaline eluent into the column from "*2.1.5*" and then plug the top of it with a stopper. Make sure both ends of the column are sealed, and then place the column on a rotator and incubate it for 10 minutes at room temperature. **Do not incubate for more than 15 minutes!**
Step 2. Collection of eluate. Set the column from "*2.1.6.1 Step 1*" on a stand vertically and place an uncapped clean centrifuge tube under it. Open the top of the column and then the bottom of it to let the solution flow into the centrifuge tube and label the solution as an eluate. Immediately neutralize the eluate with an acidic solution (1 M HCl).
Step 3. Washing and equilibrium. After collecting the eluate, immediately wash the column with 20 bed volumes of TBS buffer. Plug the bottom of the column when the TBS liquid level reaches the top of the resin.
Step 4. Resin storage: Please refer to section "*2.1.7 Step 3. Storage*".

2.1.6.2. Competitive elution

Step 1. Incubation. Add 5 bed volumes of competitive peptide eluent into the column from "*2.1.5*" and then plug the top of it with a stopper. Make sure both ends of the column are sealed, and then place the column on a rotator and incubate it for either 30 minutes at room temperature or overnight at 2°C~8°C with continuous rotation.
Step 2. Collection of eluate. Set the column from "*2.1.6.2 Step 1*" on a stand vertically and place an uncapped clean centrifuge tube under it. Open the top of the column and then the bottom of it to let the solution flow through into the centrifuge tube and label the solution as an eluate. After finishing the collection, plug the bottom of the column for further regeneration.
Step 3. Regeneration. The resin must be regenerated using an alkaline elution buffer to maintain its reusability. Please refer to "*2.1.7 Regeneration*" for the regeneration instructions.

2.1.7. Regeneration

The resin can be regenerated and reused for at least four cycles. With a proper maintenance, it can be reused for up to ten times with a slight decrease in its binding capacity.

Step 1. Regeneration. Add 10 bed volumes of the prepared regeneration solution from "*2.1.1 Table 2*" into the column and then plug the top of it with a stopper. Place the column on a rotator and incubate for 10 minutes at room temperature. **Do not incubate for more than 15 minutes!** Then, open the top of the column and then the bottom of it to drain out the regeneration solution.

Step 2. Washing. After draining out the regeneration solution, wash the column with 20 bed volumes of TBS buffer. Plug the bottom of the column when the TBS solution level reaches the top of the resin. **Keep the resin wet throughout the entire process**.

Step 3. Storage. After finishing the washing step, add 2 bed volumes of prepared TBSP from "*2.1.1 Table 2*" into the column, and then plug the top of it with a stopper. Place the column on a vortex mixer and mix it thoroughly. Store the resin at 2°C~8°C. **Do not store the resin at 0°C or below 0°C to avoid freezing the resin. Keep the resin wet for the next use.**

2.2. Immunoprecipitation of DYKDDDDK-tagged protein, IP

To perform immunoprecipitation on a small sample volume (<1 ml cell lysate), the following instructions can be used as a reference.

2.2.1. Equipment and reagents needed but not supplied

- Micropipettes
- Centrifuge tubes
- Vortex mixer
- Rotator
- Water bath
- Protease inhibitor reagents
- Buffers, see Table 3

Table 3. Buffers required for the immunoprecipitation of DYKDDDDK-tagged protein

Purpose	Solution Abbreviation	Recommend Formulation
Cell lysis	Tris-buffered saline (TBS)	50 mM Tris-HCl, 150 mM NaCl, pH 7.4
	Home-made or purchased commercial product	
Equilibrium	Tris-buffered saline (TBS)	50 mM Tris-HCl, 150 mM NaCl, pH 7.4
Washing	Tris-buffered saline with Tween 20 (TBST)	50 mM Tris-HCl, 150 mM NaCl, pH 7.4, 0.05% Tween 20
Elution	SDS-PAGE loading buffer	10 mM Tris-HCl (pH 6.8), 10% Glycerol, 0.016% bromphenol blue
	Alkaline eluent	100 mM Tris, pH 12.0
	Competitive peptide eluent	0.15-0.4 mg DYKDDDDK peptide/ml in TBS

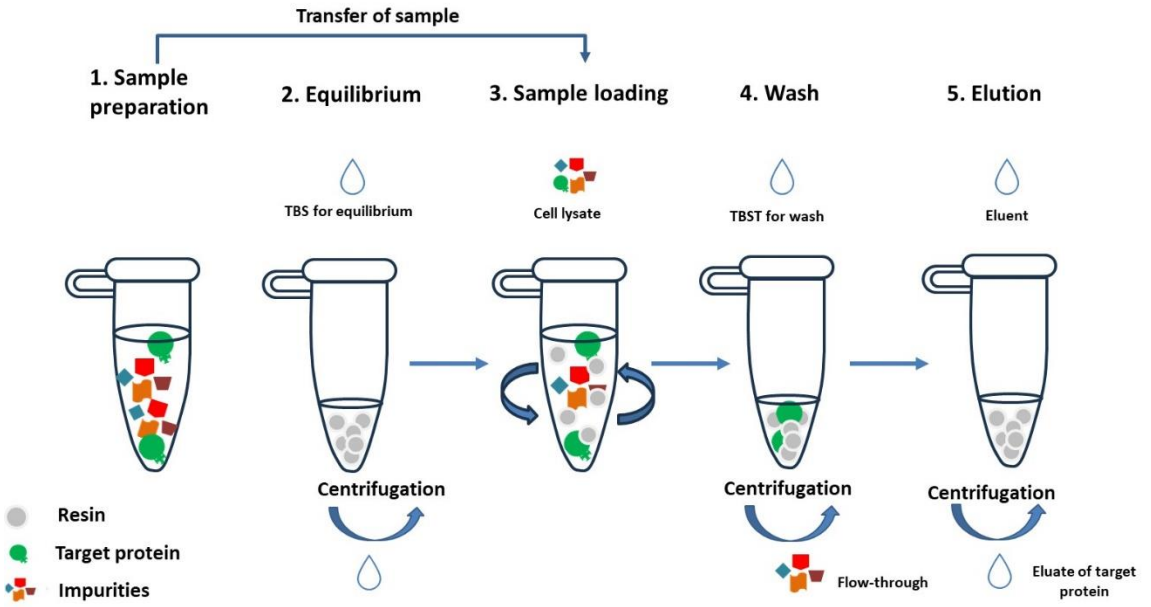


Figure 2. Flow chart of immunoprecipitation of DYKDDDDK-tagged protein

2.2.2. Sample preparation

Please refer to the section "*2.1.2 Sample preparation*".

2.2.3. Equilibrium

Step 1. Preparation of resin slurry. Use a rotator to mix the sealed resin for 5 minutes, until the resin forms a homogeneous suspension.

Step 2. Packing the resin. Prepare an uncapped clean centrifuge tube with a proper size (e.g., 1.5 ml or 2 ml). Use a pipette tip with a diameter greater than 1 mm (the tip can be cut by approximately 1-2 mm off with a pair of clean scissors) to aspirate the homogeneous resin suspension into the centrifuge tube and centrifuge it for 1 minute at 1000 g. Then take out the supernatant with a pipette and discard it. **Do not take out the resin!** Record the volume of settled resin as the bed volume.

Step 3. Equilibrium. Add 10 bed volumes of TBS buffer into the centrifuge tube, and then centrifuge it for 1 minute at 1000 g. Take out the supernatant with a pipette and discard it. .**Do not take out the resin!** Repeat "*2.2.3 Step 3*" process once.

2.2.4. Sample loading

Step 1. Transfer of the sample. Aspirate the prepared sample from "*2.2.2 Sample preparation*" and transfer it to the centrifuge tube, then cover the cap of it.

Step 2. Binding of target protein. Place the capped centrifuge tube on a rotator and mix it for 1 hour at room temperature.

Step 3. Collection of the supernatant. After incubation, centrifuge the tube for 1 minute at 1000 g. Open the cap of the tube, take out the supernatant with a pipette and transfer it to a new centrifuge tube for the further use. **Do not take out the resin**!

2.2.5. Wash away of the impurities

After the collection of the supernatant, add 10-25 bed volumes of TBST into the centrifuge tube containing the resin and cover the cap of it. Place the tube on a rotator and mix it for 5 minutes at room temperature. Centrifuge the tube for 1 minute at 1000 g and then open the cap of it, then take out the supernatant with

a pipette and discard it. **Do not take out the resin**! Repeat the 2.2.5 three times.

2.2.6. Elution

Depending on the variations of the target protein and downstream applications, different elution methods can be selected. The following elution instructions are provided for reference.

2.2.6.1. SDS-PAGE loading buffer elution

Add an equal bed volume of SDS-PAGE sample buffer into the centrifuge tube from "*2.2.5*". Cover the cap of the tube and then place it on a vortex mixer to mix the solution thoroughly. Then, place the tube in a water bath and heat it for 5 minutes to 10 minutes at 90°C~100°C. Then, centrifuge the tube for 1 minute at 1000 g. Collect the supernatant into a clean tube for the further use. **Do not take out the resin**!

2.2.6.2. Competitive elution

Add 5 bed volumes of competitive peptide eluent into the centrifuge tube from "*2.2.5*". Cover the cap of the tube and then place it on a rotator and incubate it for 30 minutes at room temperature. Then, centrifuge the tube for 1 minute at 1000 g and collect the supernatant for further use. **Do not take out the resin**!

2.2.6.3. Alkaline elution

Add 10 bed volumes of alkaline eluent into the centrifuge tube from "*2.2.5*". Cover the cap of the tube and then place it on a rotator and incubate it for 10 minutes at room temperature. Then, centrifuge the tube for 1 minute at 1000 g and collect the supernatant. Immediately neutralize the supernatant with an acidic solution (1 M HCl).

2.3. Sample analysis

The target protein can be simply determined by Western Blotting or SDS-PAGE. GenScript is providing a one-stop protein analysis solution for this application. Here are some related protein analysis products:

Cat. No.	Product name
M00653	SurePAGE™, Bis-Tris, 10x8, 4-12%, 12 wells
M00624-250	Broad Multi Color Pre-Stained Protein Standard
M00516	PAGE-MASTER Protein Standard (for SDS-PAGE)
M00521	WB-MASTER Protein Standard
MM1397-500	PAGE-MASTER Protein Standard Plus
M00676-10	4X LDS Sample Buffer
MB01015	5X Sample Buffer
L00657	eStain L1 Protein Staining Device
L00686	eBlot® L1
L00816	eZwest Western Blotting Device
L00780	GenBox Mini Electrophoresis tank

3. Reagent Compatibility

During the cell lysis process, if certain chemical reagents are used, the binding between the target protein and the resin may be influenced. The table of **Reagent Compatibility** listed the tolerable concentration of commonly used reagents for Anti-DYKDDDDK Affinity Resin Easy. Please refer to this table during the design of the whole experiment.

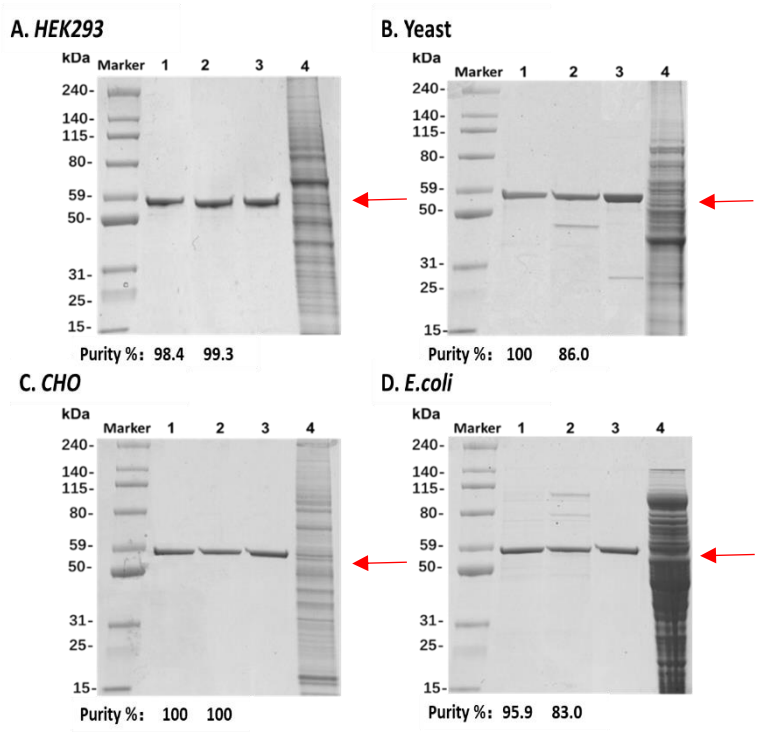
Table 4. Reagent Compatibility

Reagent type	Reagent	Maximum tolerated concentration	Effect
Chelating reagents	EDTA	10 mM	High concentration of chelating reagents can reduce the purification efficiency and leading to a low recovery of target protein.
Reducing reagents	TCEP	5 mM	Reducing reagents can disrupt the disulfide bonds in antibodies on the resin and will decrease the binding capacity of it. These reagents should be avoided or kept at low concentrations during the purification process.
	β-ME	150 mM	
	DTT	100 mM	
Surfactants	Tween 20	15%	High concentration of surfactants can reduce the purification efficiency and leading to a low recovery of target protein.
	Triton X-100	10%	
	Triton X-114	15%	
	NP-40	10%	
Denaturants	SDS	Not recommended	High concentration of denaturants can denature the target protein.
	GuHCl	0.5 M	
	Urea	2 M	
Other reagents	Glycerol	40%	High concentration of glycerol can interfere the binding of the target protein and the resin.
Salts	$(NH_4)_2SO_4$	20%	High concentration of salts can reduce the purification efficiency and leading to a low recovery of the target protein.
	KCl	1 M	
	NaCl	1 M	

4. Case Studies

4.1. Purification of DYKDDDDK-tagged protein

4.1.1. Purification of DYKDDDDK-tagged protein from various lysates



Lanes:

1. Eluate purified by GenScript resin

2. Eluate purified by Competitor-S resin

3. DYKDDDDK-tagged protein (~55 kDa)

4. Whole cell lysate

The red arrows indicate the bands of the target protein.

Figure 3. The DYKDDDDK-tagged proteins from cell lysates of various expression systems were captured by GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No. L00907) and Competitor-S resin, respectively. The captured proteins were eluted by 0.4 mg/ml MDYKDHDGDYKDHDIDYKDDDDK peptide in TBS, and then were analyzed by SDS-PAGE. The results showed that GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No. L00907) efficiently captured DYKDDDDK-tagged proteins from cell lysates of various expression systems. Especially, GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No. L00907) significantly outperforms the competing product when purifying the target protein from yeast and *E. coli* lysates.

4.1.2. Regeneration performance of Anti-DYKDDDDK Affinity Resin Easy

1) Elution with DYKDDDDK peptide

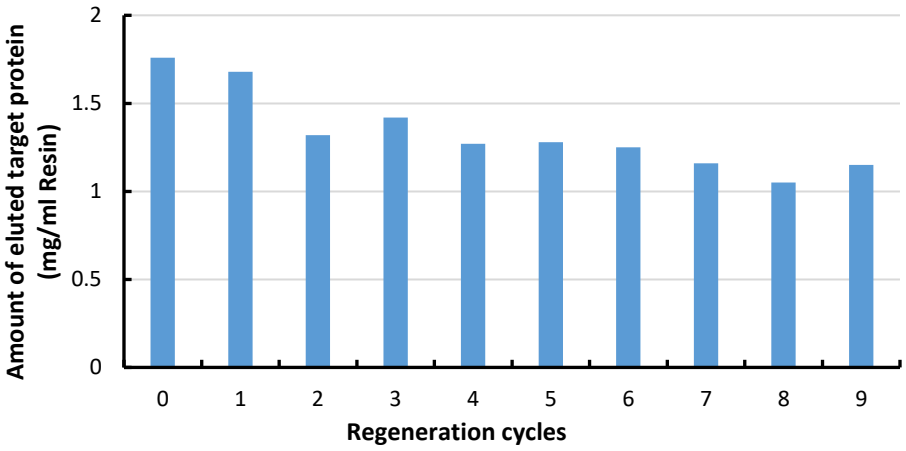


Figure 4. Purify the DYKDDDDK-tagged protein by GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No. L00907). The target protein was eluted by 0.1 mg/ml DYKDDDDK peptide in TBS. Then, the resin was regenerated by the regeneration solution for repeated use. The coefficient of variation (CV) of the amount of eluted target protein was less than 20% for 10 purification processes in total, indicating that GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No. L00907) is capable of multiple repeated use with relatively high consistency when eluted by DYKDDDDK peptide.

2) Elution with MDYKDHDGDYKDHDIDYKDDDDK peptide

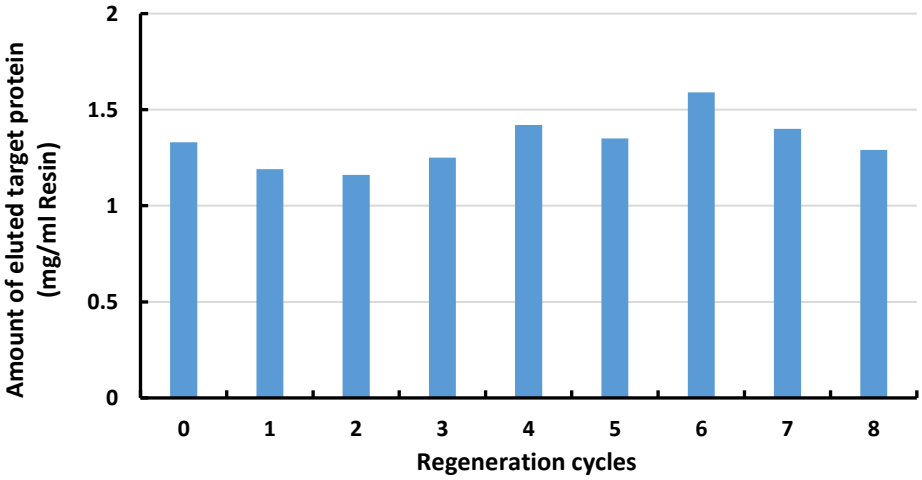
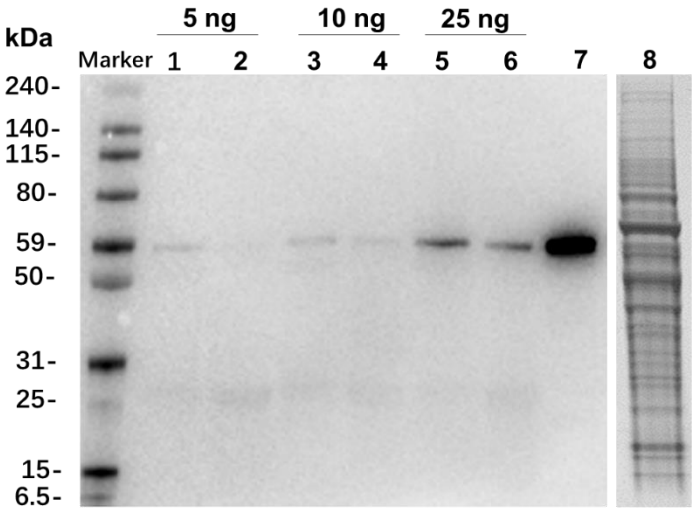


Figure 5. Purify the DYKDDDDK-tagged protein by GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No. L00907). The protein was eluted by 0.4 mg/ml MDYKDHDGDYKDHDIDYKDDDDK peptide in TBS. Then, the resin was regenerated by the regeneration solution for repeated use. The coefficient of variation (CV) of the amount of eluted target protein was less than 20% among 9 purification processes in total, indicating that GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No. L00907) is capable of multiple repeated use with relatively high consistency as well when eluted by MDYKDHDGDYKDHDIDYKDDDDK peptide.

4.2. Immunoprecipitation for DYKDDDDK-tagged protein

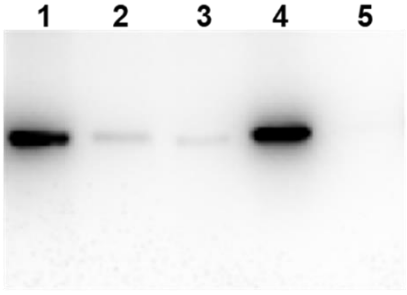
4.2.1. Efficient capture of DYKDDDDK-tagged protein



Lanes:
1/3/5. IP Eluate by GenScript resin
2/4/6. IP Eluate by Competitor-S resin
7. DYKDDDDK-tagged protein (~55 kDa)
8. Whole *HEK293* lysate

Figure 6. Different amounts (5/10/25 ng) of DYKDDDDK-tagged protein (~55 kDa) were spiked to 0.5 ml of *HEK293* lysis buffer, and captured by GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No. L00907) and Competitor-S resin, respectively. After washing step, the resin (containing the target protein) was then eluted by SDS-PAGE sample loading buffer, followed by a Western Blotting analysis of the eluted protein. The data showed that GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No. L00907) outperforms the competing product in sensitivity for immunoprecipitation applications.

4.2.2. Low binding of HA-tagged fusion protein



Lanes:
1. HA-tagged protein (~20 kDa)
2. Competitor-S resin
3. Competitor-T resin
4. Competitor-ST resin
5. GenScript resin

Figure 7. 10 µg of HA-tagged protein was incubated with GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No.L00907) and other competitor products, respectively. The proteins were eluted by SDS-PAGE sample loading buffer, then analyzed by Western blotting. Data showed that GenScript Anti-DYKDDDDK Affinity Resin Easy (Cat. No. L00907) exhibited extremely low nonspecific binding to the HA-tagged protein and outperforms the competing products.

5. Troubleshooting

Problems	Possible Causes	Solutions
Problem 1: A large quantity of DYKDDDDK -tagged proteins was found in the flow-through.	Insufficient reaction time	Extend the reaction time; incubate for 1 hour at room temperature or overnight at 2°C~8°C.
	The resin was not fully re-suspended during the incubation process	Increase the speed and time of rotation appropriately during the incubation of sample and resin to improve the binding efficiency.
	Resin was overloaded	Reduce the loading volume of sample or increase the amount of resin.
	The explosion of the DYKDDDDK-tag was\s not enough for an effect binding with the resin	Add a small amount of denaturant to the sample to expose the epitope of the tag (which may require dialysis before binding to the resin) or fuse the DYKDDDDK tag to the opposite end of the target protein.
	Resin was not regenerated properly	Perform resin regeneration step before loading the sample
	Insufficient binding and decreased recovery of target protein due to certain chemical reagents used in the process	Refer to the reagent compatibility table to evaluate if an excess amount of certain chemical reagent is used. If so, perform a TBS dialysis on the sample before the purification process.
Problem 2: The DYKDDDDK -tagged protein was absent in the eluate.	A large quantity of DYKDDDDK-tagged proteins was found in the flow-through.	Refer to the possible cause and solution in “Problem 1”.
	Degradation of the target protein	1. Use freshly prepared samples; 2. Perform the purification at a lower temperature, e.g., 2°C~8°C, and extend the incubation time of the target protein and resin properly; 3. Add a protease inhibitor during cell lysis and binding steps.
	The target protein was not completely eluted.	1. For the competitive elution: increase the peptide concentration and extend the elution time to 1 h; 2. For alkaline elution: increase the amount of NaCl (< 2 M), EDTA (< 20 mM), GuHCl (< 0.25 M) to the alkaline elution buffer.
	No expression of the target protein	Confirm the presence of the DYKDDDDK-tagged target protein in the cell lysate by a Western blotting analysis before the purification.
	The expression level of the target protein was extremely low	1. Detect the presence of the target protein in the cell lysate; 2. If the target protein was found in the cell lysate with extremely low concentration, increase the sample volume for loading or optimize expression conditions to improve the

Problems	Possible Causes	Solutions
		expression level of target protein.
Problem 3: Multiple protein bands were detected in the eluate.	The target protein was unstable at room temperature.	Purify the target protein at lower temperatures, e.g., 2°C~8°C, and extend the incubation time of the target protein and resin properly.
	During the purification process, protein degradation caused by proteases	Add protease inhibitors to the sample.
	Non-specific binding	1. Increase the number of wash cycles or extend the washing time (rotary incubation for 5 minutes~10 minutes); 2. Add a small amount of Tween 20 (< 15%) or NaCl (< 1 M) to the washing solution.

6. Related Products

Cat. No.	Product Name
Z03695	Benz-Neburase™, tag-free
A00187	THETM DYKDDDDK Tag Antibody, mAb, Mouse
A01428	THETM DYKDDDDK Tag Antibody [HRP], mAb, Mouse
A01429	THETM DYKDDDDK Tag Antibody [Biotin], mAb, Mouse
A01632	THETM DYKDDDDK Tag Antibody [FITC], mAb, Mouse
A00170	DYKDDDDK-tag Antibody, pAb, Rabbit
RP10586	DYKDDDDK Peptide
RP21087	MDYKDHDGDYKDHDIDYKDDDDK peptide
M00653	SurePAGE™, Bis-Tris, 10x8, 4-12%, 12 wells
M00624-250	Broad Multi Color Pre-Stained Protein Standard
M00516	PAGE-MASTER Protein Standard (for SDS-PAGE)
M00521	WB-MASTER Protein Standard
MM1397-500	PAGE-MASTER Protein Standard Plus
M00676-10	4X LDS Sample Buffer
MB01015	5X Sample Buffer
L00657	eStain L1 Protein Staining Device
L00686	eBlot® L1
L00816	eZwest Western Blotting Device
L00657	eStain L1 Protein Staining Device

For laboratory research use only. Direct human use, including taking orally and injection and clinical use are forbidden.

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Manufacturer: Nanjing GenScript Biotech Co., Ltd. No. 28 Yongxi Road, Jiangning District, Nanjing, Jiangsu, China

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