Anti-DYKDDDDK G1 Affinity Resin **Cat. No. L00432**

Technical Manual No. TM0634 **Version 03222012**

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I. Description

DYKDDDDK or FLAG® tag is an octapeptide tag that can be added to a protein using recombinant DNA technology. It can be fused to the N-terminus or C-terminus of a target protein to facilitate detection and purification. **GenScript Anti-DYKDDDDK G1 Affinity Resin (Cat. No. L00432)** is designed for the purification of DYKDDDDK-tagged protein from commonly used protein expression systems including bacteria, yeast and mammalian cells. The DYKDDDDK-tagged protein in the cell lysate can specifically bind to the anti-DYKDDDDK monoclonal antibodies coupled to the resin. Nonspecific bindings are eliminated by stringent washing steps, and the target protein is finally eluted with high recovery rate. Table 1 lists the main characteristics of Anti-DYKDDDDK G1 Affinity Resin.

Table 1. Characteristics of Anti-DYKDDDDK G1 Affinity Resin

Product content	50% settled resin in TBS with 0.02% sodium azide
Matrix	4% cross-linked agarose
Average bead size	90 µm
Ligand	Mouse monoclonal antibody against DYKDDDDK tag, clone G1
Binding capacity	Approximately 1 mg DYKDDDDK-tagged protein per ml settled resin
Storage and stability	Store at 2-8 °C for up to 12 months. Do not freeze the resin.
Resin reuse	The resin can be recycled for at least 4 times. If maintained properly, the resin can be reused 10 times with minimum loss of binding capability.
Elution method	Acid, alkaline, neutral, peptide competitive and SDS-PAGE loading buffer elutions
Reagents compatibility	Compatible with commonly used reagents at certain concentrations

II. Equipments and Reagents Required But Not Supplied

- Distilled water
- Micropipettors
- Microcentrifuge tubes
- Vortex mixer
- Reagent reservoirs
- Empty Columns
- Serological pipettes (5 ml, 10 ml)
- Cell lysis buffer

- Protease inhibitor reagents
- Buffers (Table 2 lists the necessary buffers for use of Anti-DYKDDDDK G1 Affinity Resin.)

Table 2. Buffers necessary for the use of Anti-DYKDDDDK G1 Affinity Resin

Purpose	Buffer	Formulation
Equilibration and Washing	Tris-buffered saline, TBS	50 mM Tris-HCl, 150 mM NaCl, pH 7.4
Elution	Alkaline elution buffer pH 12.0	0.1 M Tris, 0.5 M NaCl, pH 12.0
	Alkaline elution buffer pH 10.5	0.1 M Tris, 0.5 M NaCl, pH 10.5
	Acid elution buffer	0.1 M glycine HCl, pH 3.5
	Neutral elution buffer	3-4 M NaCl in distilled water
	Competitive elution buffer	DYKDDDDK or FLAG® peptide in TBS with concentration of 100-500 µg/ml
	PAGE gel sample buffer	0.01 M Tris-HCl (pH 6.8), 10% Glycerol, 0.016% Bromophenol Blue
Regeneration of used resin	Regeneration buffer 1	0.1 M Tris HCl, 0.5 M NaCl, pH 8.0
	Regeneration buffer 2	0.1 M sodium acetate, 0.5 M NaCl, pH 4.0

III. Instruction for Use

1. Sample Preparation

For optimal results, follow the recommendations below for sample preparation.

1) Prepare sample according to protein characteristics, optimize lysis conditions to minimize factors interfering with protein binding (See *Reagent Compatibility Table*).
2) For binding step of DYKDDDDK-tagged protein, apply proper salt (NaCl) concentration at neutral pH.
3) Sample should not contain any insoluble particles. Filter the sample or centrifuge with high speed for 10-15 min at 4 °C to remove the insoluble materials before binding procedure.
4) To prevent protein degradation during the purification process, perform sample preparation on ice and/or add protease inhibitors to the sample during cell lysis.
5) During cell lysis, add endonucleases to reduce sample viscosity caused by the release of chromosomal DNA or RNA.
6) Avoid frequent freezing-thawing cycles. Make lysate/sample aliquots and store at -80°C.

2. Resin Preparation

1) Place an empty column on a firm support; rinse the column once with TBS.
2) Thoroughly resuspend the resin by gentle inversion and immediately load appropriate volume of the slurry into the column. Wide bore pipette tips are recommended for easy resin slurry transfer.
3) Equilibrate the resin by washing with 3 bed volumes of TBS, repeat for a total of 3 times. Allow the buffer to drain from the column by gravity; do not let the resin run dry.

3. Binding Procedures

The Anti-DYKDDDDK G1 Affinity Resin can purify DYKDDDDK-tagged protein by different methods including column, batch or immunoprecipitation (IP). If the sample volume is around 50ml with less than 4 mg DYKDDDDK-tagged protein, column binding format with 2-4 ml of settled resin is recommended. For larger sample volume, batch binding format is recommended for quick and efficient purification. For small

volume of sample with low protein expression level, apply the immunoprecipitation method (See *Section III.7 Immunoprecipitation of DYKDDDDK-tagged Protein*)

3.1 Column Chromatography

1) Load the prepared sample onto the column under gravity flow at room temperature. Attach a buffer reservoir to the bottom of the column for loading large volume. Collect and reload flowthrough several times for maximal binding. Lower flow rate may facilitate better target protein binding.
2) Wash the column with 10 - 20 bed volumes of TBS to reduce non-specific bindings.
3) Allow the column to drain completely and proceed to elution procedure. (See *Section III.4 Elution of DYKDDDDK-tagged protein*)

3.2 Batch Binding

1) Resuspend prepared resin (as described in *Section III.2*) in the column completely with prepared sample containing target protein.
2) Transfer the resuspended resin to the remaining sample. Incubate the sample with Anti-DYKDDDDK G1 Affinity Resin at room temperature for at least 30 min with rotation.
3) After incubation, load the sample with resin onto another column to collect the resin. Preserve the flow through of the sample for further use.
4) Wash the resin with 10-20 bed volumes of TBS to remove any non-specific binding.
5) Allow the column to drain completely and proceed to elution procedure. (See Section *III.4 Elution of DYKDDDDK-tagged protein*)

Notes:

1. The binding time can be extended empirically. The incubation can be done overnight at 4°C with end-to-end rotation. To prevent protein degradation, protease inhibitor should be added to the sample.
2. Preserve the flow through after binding for further SDS-PAGE analysis or Western Blot detection to determine the binding efficiency of the DYKDDDDK-tagged protein.

4. Elution of DYKDDDDK-tagged Protein

Five elution methods are recommended according to protein characteristics and downstream applications. The selected elution method should preserve protein structure integrity and biological function, as well as to be compatible with downstream applications. To achieve optimal results, multiple elution methods could be used in combination. All the methods can be carried out at room temperature; lower temperature may be necessary depending on special requirements. After the final wash step, immediately load elution buffer onto the column. See *Section II, Equipment and Reagents Required But Not Supplied*, for recipes of recommended elution buffers.

4.1 Elution with alkaline elution buffer , pH 12.0

Elute the bound DYKDDDDK-tagged protein with 6 bed volumes of Alkaline elution buffer (pH 12.0), into vials containing 1/20 bed volume of 1 M HCl to neutralize the eluate. For example, for 1 ml settled resin, use 6 × 1 ml elution buffer to elute sequentially and collect the eluate with six vials containing 50 µl 1M HCl, respectively. Do not leave the column in the alkaline elution buffer for more than 15 minutes; re-equilibrate the column immediately after elution. (See *Section III.5*)

4.2 Elution with alkaline elution buffer , pH 10.5

This buffer is at lower pH (10.5) than alkaline elution buffer (pH 12), which may help preserve bioactivity of target protein. In some cases, this procedure may not recover as much target DYKDDDDK-tagged protein as by alkaline elution buffer, pH 12.0.

Elute the bound target protein with 6 bed volumes of alkaline elution buffer (pH 10.5), into vials containing 1/40 bed volume of 1 M HCl. Do not leave the column in the alkaline elution buffer for more than 15 minutes; re-equilibrate the column immediately after elution. (See *Section III .5 Re-equilibration)*

4.3 Elution with 0.1 M acid elution buffer

Elute the bound target protein with 6 bed volumes of acid elution buffer (pH 3.5), into vials containing 1/20 bed volume of 1 M Tris, pH 9.0. Do not leave the column in the acid elution buffer for more than 15 minutes; re-equilibrate the column immediately after elution.

4.4 Elution with competitive elution buffer

Competitive binding with added DYKDDDDK or FLAG® peptide can elute bound target protein. The concentration of DYKDDDDK or FLAG® peptide in competitive elution buffer may vary from 100-500 µg/ml. Load 2-3 bed volumes of competitive elution buffer into the column by gravity flow; when there is about 1 bed volume of elution buffer left on top of the resin, cap the bottom of column and incubate at room temperature for 30-60 minutes; open the end of the column and collect the eluate.

4.5 Elution with neutral elution buffer

High concentration of NaCl can be used to elute DYKDDDDK-tagged proteins. Sequentially load 5x1 bed volume of 3 M NaCl buffer (pH 7.4) onto the column and collect the eluate. The NaCl concentration could be increased up to 4 M if 3 M NaCl cannot completely elute target protein.

5. Re-equilibration

After elution, the resin should be washed with TBS buffer to remove residual elution buffer that may denature the G1 antibodies immobilized on the resin. It is recommended to wash the resin 3 times each with 5-10 bed volumes of TBS buffer. After the final wash, allow TBS buffer to drain completely before adding 2 bed volumes of TBS to the resin for next use.

6. Regeneration of Anti-DYKDDDDK G1 Affinity Resin

The Anti-DYKDDDDK G1 Affinity Resin can be reused several times to purify the same protein without regeneration. If the target DYKDDDDK-tagged protein to be purified is different, the resin must be regenerated using the following protocol:

6.1 Wash the resin with 2 bed volumes of 0.1 M Tris HCl, 0.5 M NaCl, pH 8.0.

6.2 Wash the resin with 2 bed volumes of 0.1 M sodium acetate, 0.5 M NaCl, pH 4.0.

6.3 Re-equilibrate the resin with 3-5 bed volumes of TBS.

6.4 For long-term storage, the resin should be stored in TBS containing 0.02% sodium azide at 2-8°C.

7. Immunoprecipitation of DYKDDDDK-tagged Protein

For target proteins to be purified from a small volume of starting sample (e.g., 1–2 ml of cell lysate), the immunoprecipitation procedure can be applied.

7.1 Resuspend the resin to form a uniform slurry and transfer 40-100 µl of the slurry into a 1.5 ml vial.

Note: Wide bore pipette tips are recommended for transferring resin slurry.

7.2 Add 500 µl TBS into the vial and gently mix the resin. Centrifuge at 6,000 × g for 30 seconds, remove supernatant carefully. Repeat this step two more times. Carefully remove as much supernatant as possible after each wash without disturbing the settled resin.

7.3 Add sample to the washed resin. If the sample volume is less than 1 ml, add lysis buffer or TBS to make the total sample volume to 1 ml.

7.4 Mix by end-to-end rotation on a tube rotator for at least 1 hour at room temperature.

Note: For optimal elution, the incubation time can be extended and lower incubating temperature may be required also.

7.5 Centrifuge at 6,000×g for 30 seconds. Carefully remove supernatant and add TBS to wash the resin three times. Remove as much supernatant as possible without disturbing the resin, proceed to elution procedure.

7.6 Extreme pH condition or DYKDDDDK peptide can be applied to elute the DYKDDDDK-tagged protein. The resin bound with target protein also can be applied directly for SDS-PAGE analysis. Choose one of the following elution methods according to the characteristics of target protein and downstream application.

1) Elution with alkaline elution buffer, pH 12.0

Add 120-300 µl (3 column volumes) of alkaline elution buffer into the washed resin and use a wide bore pipette tip to gently resuspend the resin. Incubate at room temperature for 5 minutes, mix gently by tapping the tube once or twice during the incubation period. After incubation, centrifuge at 6,000 × g for 30 seconds. Carefully transfer supernatant into a new vial containing 5-15 µl 1 M HCl for further application.

2) Elution with acid elution buffer

Add 120-300 µl (3 column volumes) of acid elution buffer into the washed resin and use a wide bore pipette tip to gently resuspend the resin. Incubate at room temperature for 5 minutes, mix gently by tapping the tube once or twice during the incubation period. After incubation, centrifuge at 6,000 × g for 30 seconds. Carefully transfer supernatant into a new vial containing 5-15 µl 1 M Tris, pH 9.0 for further application.

3) Elution with competitive elution buffer

Add 120-300 µl (3 column volumes) of 100 µg/ml DYKDDDDK peptide elution buffer into the washed resin and use a wide bore pipette tip to gently resuspend the resin. Incubate at room temperature for 5 minutes, mix gently by tapping the tube once or twice during the incubation period. After incubation, centrifuge at 6,000 × g for 30 seconds. Carefully transfer supernatant into a new vial for further application.

4) Elution with neutral elution buffer

Add 120-300 µl (3 column volumes) of 3M NaCl, pH 7.4 into the washed resin and use a wide bore pipette tip to gently resuspend the resin. Incubate at room temperature for 5 minutes, mix gently by tapping the tube once or twice during the incubation period. After incubation, centrifuge at 6,000 × g for 30 seconds. Carefully transfer supernatant into a new vial for further application.

5) Elution with PAGE gel sample buffer

In order to minimize the denaturation and elution of the G1 antibodies immobilized on the resin, no

reducing reagents (β-mercaptoethanol or DTT) should be included in the sample buffer. Reducing reagents will dissociate the heavy and light chains of the G1 antibody. If reducing condition are absolutely necessary, a reducing agent may be added. Please refer to *Section IV Reagents Compatibility, Table 3.*

Add 40-100 µl (1 column volume) of PAGE gel sample buffer to the washed resin and mix well. Boil for 3 minutes and centrifuge at 8,000 × g for 30 seconds. Carefully transfer supernatant into a new vial.

IV. Reagents Compatibility Table

The tolerable concentration of listed reagents are tested by addition of listed reagents at indicated concentrations.

Table 3. Reagents Compatibility

Reagent	Maximum Tolerable concentration	Note
EDTA	5 mM	Higher concentration of chelating agent will reduce purification efficiency with less target protein recovery.
β-ME	100 mM	Reducing agents will reduce disulfide bonds in the G1 antibody on the resin. Avoid reducing agents or keep at low concentration (<10mM) during purification process. When the reducing agents reach the maximum tolerable concentration mentioned here, there will be a band of antibody heavy chain (～50 kDa) in SDS-PAGE analysis. The resin CANNOT BE REUSED if samples containing higher concentration of reducing reagents are applied to the resin.
DTT	80 mM	
Tween 20	5%	The concentration of detergents should not exceed 5%.
Triton X-100	5%	
SDS	Not suggested	This detergent will denature the G1 antibody on the resin.
NP-40	4%	Higher concentration will reduce purification efficiency with less target protein recovery.
GuHCl	0.3 M	Chaotropic reagents will denature the target DYKDDDDK-tagged protein. Do not exceed 0.3 M GuHCl or 1.5 M Urea.
Urea	1.5 M	
Glycerol	20%	Higher concentration will interfere with the binding of DYKDDDDK-tagged protein.
NaCl	0.5 M	Higher concentration will reduce purification efficiency with less target protein recovery.

V. Application Examples

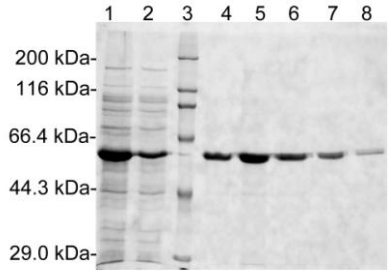
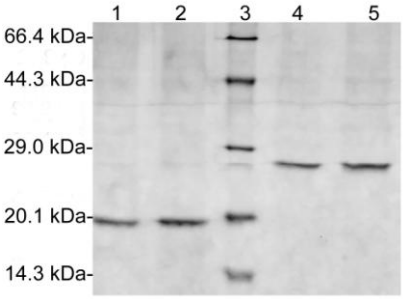
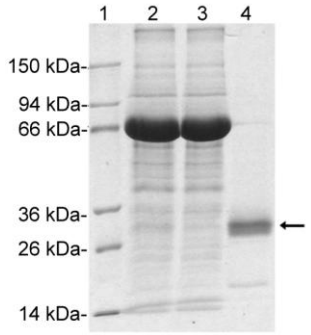
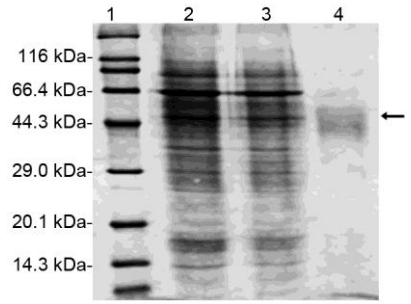
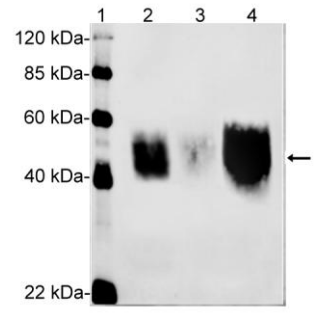
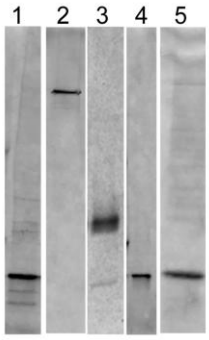
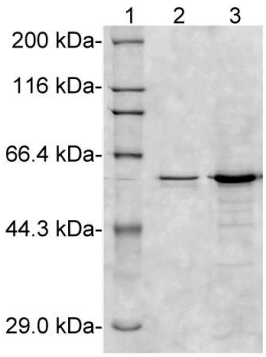
Description	Figure	Figure legend
Figure 1. Purification of DYKDDDDK-tagged protein from *E. coli* lysate		Lanes: 1. Whole cell lysate 2. Flow through 3. Protein marker 4-8. Eluate of target DYKDDDDK-tagged protein (M.W. 60.4 kDa)
Figure 2. Purification of N-terminus and C-terminus DYKDDDDK -tagged proteins		Lanes: 1-2. Eluate of N-terminus DYKDDDDK-tagged protein (M.W. 19.2 kDa) 3. Protein marker 4-5. Eluate of C-terminus DYKDDDDK-tagged protein (M.W. 26.4 kDa)
Figure 3. Purification of DYKDDDDK-tagged protein from mammalian cells		Lanes: 1. Protein marker 2. Supernatant from cultured CHO cells 3. Flow through after binding 4. Eluate of target DYKDDDDK -tagged protein (M.W. 27.6 kDa)
Figure 4. Purification of DYKDDDDK-tagged glycoprotein from mammalian cells	 	The upper figure shows SDS-PAGE analysis result and the lower figure shows Western blot result. Lanes: 1. Protein marker 2. Supernatant from CHO cells expressing target glycoprotein 3. Flow through after binding 4. Eluate of target DYKDDDDK-tagged protein (M.W. 50.0 kDa)

Figure 5. Purification of DYKDDDDK-tagged proteins using different elution methods.		Lanes: 1. Protein #1 (M.W. 16.8 kDa) by competitive elution using 300 µg/ml DYKDDDDK peptide. 2. Protein #2 (M.W. 54.6 kDa) by neutral elution with 3M NaCl 3. Protein #3 (M.W. 27.6 kDa) by acid elution, pH 3.5 4. Protein #1 (M.W. 16.8 kDa) by alkaline elution, pH 10.5 5. Protein #1 (M.W. 16.8 kDa) by alkaline elution, pH 12
Figure 6. Benchmark with Sigma-Aldrich's ANTI-FLAG M2 Affinity Gel		Side-by-side comparison of DYKDDDDK-tagged protein purification with GenScript's Anti-DYKDDDDK G1 Affinity Resin and Sigma-Aldrich's ANTI-FLAG M2 Affinity Gel, from same staring material following manufacturer suggested procedures. Lanes: 1. Protein marker 2. Purification from M2 gel (pH 3.5 elution) 3. Purification from G1 resin (pH 12.0 elution)

VI. Troubleshooting

Problem	Possible Cause	Solution
Large amount of DYKDDDDK-tagged protein found in the flow through	Binding time is not enough	If using batch method, increase the binding time experimentally; If using column method, use a lower flow rate when loading samples.
	Column is overloaded	Reduce the amount of the sample added to the resin or increase the amount of resin.
	DYKDDDDK-tag is not accessible to resin.	Expose the epitope tag by adding low amount of denaturant to the protein extract (dialysis may be needed before applying onto resin), or fuse DYKDDDDK tag to the other terminus of the target protein.
	Resin has not been regenerated since last purification.	Perform resin regeneration procedure prior to binding.
	Reagent compatibility problem	Dialyze the sample against TBS before purification procedure.
Very few or no DYKDDDDK-tagged protein exists in the eluate.	The target protein has been degraded.	1. Use freshly prepared sample 2. Perform purification at lower temperature, such as 4 °C 3. Include protease inhibitors to the sample during cell lysis and binding steps.
	Protein is not completely eluted	Change elution methods according to the instructions in *Section III.4*.
	No target protein expressed	Confirm the presence of target DYKDDDDK-tagged protein in cell lysate with Western blot before purification.
	Very low protein expression level	1. Use larger volume of cell lysate. 2. Optimize expression conditions to raise the protein expression level.
Multiple protein bands found in the eluate.	The protein is not stable at room temperature.	Purify the target protein at lower temperature, such as 4 °C.
	Protein degradation due to proteases activity during purification process	Add protease inhibitors to cell lysate.
	Non-specific binding	1. Prepare cell lysate again. 2. Add additional wash steps.

VII. DYKDDDDK-tag Related Products

- A00187 THE™ DYKDDDDK Tag Antibody, mAb, Mouse
- A01428 THE™ DYKDDDDK Tag Antibody [HRP], mAb, Mouse
- A01429 THE™ DYKDDDDK Tag Antibody [Biotin], mAb, Mouse
- A01632 THE™ DYKDDDDK Tag Antibody [FITC], mAb, Mouse
- A00170 DYKDDDDK-tag Antibody, pAb, Rabbit
- RP10586 DYKDDDDK Peptide
- M0005 DYKDDDDK Lysates

GenScript USA Inc.
860 Centennial Ave.,
Piscataway, NJ 08854
Tel: 1-877-436-7274, 1-732-885-9188
Fax: 732-210-0262, 732-885-5878
Email: product@genscript.com
Web: www.genscript.com