www.rockland.com
tech@rockland.com
+1 484.791.3823

Protocol

DYKDDDDK (FLAG®) Immunoprecipitation Kit

The DYKDDDDK (FLAG®) Immunoprecipitation Kit (KBA-319-383) is intended to provide a simple, reliable and convenient purification system for recombinant proteins containing the FLAG® epitope tag. The FLAG® epitope tag is a small but highly immunogenic peptide DYKDDDDK (N-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C), which allows fusion proteins to retain their original conformation and function. The hydrophilic character of FLAG® increases the likelihood that it will be located on the surface of the fusion protein where it is accessible to antibodies. The kit allows a rapid and efficient immunoprecipitation and elution of an active FLAG®-tagged recombinant protein in less than 2 hours. The immunoprecipitation is performed with anti-FLAG® antibody coupled to agarose beads, which are generated by covalently linking agarose to a highly specific mouse monoclonal antibody raised against FLAG®. The provided protocol is a guideline. Any procedure can be altered according to specific experimental requirements.

The DYKDDDDK (FLAG®) Immunoprecipitation Kit allows for the purification of recombinant proteins by IP containing the FLAG® epitope tag. The kit relies upon the high specificity of monoclonal antibody raised against the FLAG® epitope tag. This method is far easier and less costly than using antibodies produced against the recombinant protein itself therefore saving time and resources. Using the agarose bound antibody in this kit allows for efficient binding of FLAG® tag proteins without the need for preliminary steps or calibration. The immunoprecipitated FLAG® tag protein can be efficiently eluted from the agarose beads using a low pH elution step. The user is able to further characterize the resultant purified protein by size, post-translational modification, western blot and other assays.

I. Components

Product	Size	Storage
anti-FLAG® coupled to agarose beads	1.5 mL	4°C
FLAG® positive control lysate	0.2 mL	-20°C
2X SDS-PAGE Sample Buffer	1.5 mL	-20°C
Neutralization Buffer	2 mL	4°C
1X Lysis Buffer	50 mL	4°C
10X Wash Buffer	50 mL	4°C
Elution Buffer	10 mL	4°C
Microspin Columns	5 each	N/A
Collection Tubes	5 each	N/A

Note: The reagents in this kit are sufficient to perform 50 X 20 µL reactions. This kit is stable for at least one year when stored as indicated. Upon receipt store items 2-3 at -20°C. Store items 1 and 4-7 at 4°C. Store items 8-9 at room temperature. Individual components are stable for 3-4 weeks after dilution when stored at 4°C.

II. Equipment Required

1. Transfer pipettes or micro pipettes
2. Microcentrifuge
3. Additional tubes and/or microcentrifuge tubes with caps
4. Recombinant protein or lysate containing the FLAG® epitope tag
5. Protease cocktail inhibitor
6. Materials relating to cell culture (if necessary)
7. 0.22-um syringe filter (or equivalent)
8. Liquid nitrogen (LN2)
9. 18-gauge needle and syringe
10. SDS-PAGE gels, equipment and related materials

III. Preparation of Working Solutions & Lysates

Wash Buffer

1. We have included 50 mL of 10X PBS sufficient to produce 500 mL of 1X Wash Buffer. Prepare solution using deionized water (or equivalent).
2. Pass solutions through a 0.22-um filter prior to use.
3. Store diluted solutions at +4°C for a maximum of 3 to 4 weeks. This buffer does not contain preservative.
4. Dilute 10 mL of 10X PBS provided up to 100 mL with deionized water. Mix thoroughly.
   Note: There is no need to adjust pH.
5. The resultant buffered saline solution is ready-to-use and contains 0.01 M Sodium Phosphate, 0.14 M Sodium Chloride, pH 7.2. Chill to 2 to 8°C prior to use.

Lysis Buffer

Lysis Buffer is provided as a ready-to-use 1X solution containing 50 mM Tris Cl, 150 mM Sodium Chloride, 1mM EDTA, 1% TRITON X-100, pH 7.4. No preparation is required prior to use. Chill to 2 to 8°C prior to use.

Mammalian Cell Lysates

Note: Generally 5 x 10^6 to 1 x 10^7 cells are required to produce recombinant protein from mammalian cells in sufficient quantity for purification. Cells expressing the FLAG®-tagged recombinant protein of interest should be seeded and grown in the appropriate medium until they are 80-90% confluent.

- Harvest cells using trypsin following standard cell culture procedures.
- Collect cells by centrifugation.
- Wash the cells twice by re-suspending the cell pellet with 1X Wash Buffer and centrifuge for 5 minutes at 420 x g.
- Decant the supernatant and discard.
- Resuspend the cell pellet in Lysis Buffer at 5 x 10^6 to 1 x 10^7 cells/mL.
- The cell lysates can be frozen in liquid nitrogen and stored at -70°C prior to use. If the expression level of the FLAG® -tagged recombinant protein is relatively low, then lyse the cells at a higher concentration of

cells/mL to increase the concentration of recombinant protein. It is highly recommended to add a protease inhibitor cocktail to the lysis buffer especially if stored for future use.

1. Resuspend the cell pellet in Lysis Buffer at 5 x 10^6 to 1 x 10^7 cells/mL. Add sufficient amount of protease inhibitor cocktail to the Lysis Buffer.
2. Lyse the cells by two freeze-thaw cycles using liquid nitrogen. Alternatively, a dry ice/ethanol bath followed by immersion in a 42°C water bath may be used.
3. Shear DNA by passing the preparation through an 18-gauge needle 3–4 times.
4. Centrifuge the cell lysate for 10 min at 12,000 rpm to pellet the cellular debris. Transfer supernatant to a fresh tube.
5. Maintain on ice for immediate use or store at -70°C for future use.

Preparation of Cell Lysates from Other Sources

Researchers should follow appropriate protocols when preparing cell lysates from bacteria, yeast, and insect or plant cells. Some detergents, such as SDS, 2-mercaptoethanol, dithiothreitol (DTT), deoxycholate (DOC) or chaotropes, like guanidine HCl may reduce the affinity of anti-FLAG® antibody for FLAG®-tagged recombinant proteins. Researchers should determine the optimum cell lysis buffer conditions before performing the intended experiment.

IV. Immunoprecipitation Procedure

Note: The highly active antibody bound to agarose beads in this kit binds a maximum amount of FLAG® tagged protein that is subsequently eluted by changing the buffer conditions. Depending on the nature of the user's lysates or recombinant protein, specific conditions may be changed as necessary. All reactions can be performed at 2 to 8°C unless stated otherwise. Remember to use cold Wash Buffer and Lysis Buffer and to pre-cool centrifuges. See the troubleshooting guide for recommendations to use the FLAG® positive control lysate if necessary.

Preparing the Microspin Column for Use

1. Snap off the tip of a microspin column and add 0.2 mL of anti-FLAG® coupled to agarose beads as a slurry into the top part of the column. Note: as little as 20 µL agarose beads can be used per reaction.
2. Place the microspin column into a collection tube and centrifuge for 1 minute in a microcentrifuge precooled to 2 to 8°C.
3. Remove the microspin column from the collection tube and discard the flow-through buffer. Place the spin column back into the same collection tube.
4. Wash the agarose beads by adding 0.4 mL of 1X Wash Buffer to the microspin column. Centrifuge as before.
5. Remove the microspin column from the collection tube and again discard the flow-through buffer. Place the spin column back into the same collection tube.
6. Repeat steps 4 and 5 two (2) more times for a total of three (3) washes. Incubate the mixture for 30 minutes at 4°C, mixing as before.

Loading the Sample

1. Load 0.2 to 0.5 mL of the sample of cell lysate containing the FLAG®-tagged recombinant protein to be purified to the agarose in the microspin column.
2. Incubate for 30 min to 1 hour at room temperature. Some FLAG®-tagged recombinant proteins may require incubation overnight at 4°C for maximum binding.

3. Centrifuge for 1 minute in a microcentrifuge.
   Note: Steps 1 and 2 may be repeated up to two (2) additional times for larger volume samples until the entire sample has been applied. Be careful not to exceed the binding capacity of the agarose (0.6 mg FLAG®-tagged recombinant protein/cc agarose). Save the flow through solution from sample loading in a microcentrifuge tube for future analysis, if desired.
4. Wash the agarose beads to remove non-specific proteins by adding 0.6 mL of 1X Wash Buffer.
5. Centrifuge for 1 minute in a microcentrifuge and discard the flow-through buffer.
6. Repeat steps 4 and 5 three (3) more times for a total of four (4) washes.

Eluting the purified recombinant protein

1. Transfer the microspin column to a clean microcentrifuge tube with cap.
2. Add 0.1 mL to 0.2 mL of Elution Buffer to the microspin column. Using a pipette, suspend the anti-FLAG® coupled to agarose beads in the Elution Buffer.
3. Incubate 5 min at room temperature. No further agitation is required (see notes section).
4. Centrifuge as before to collect the eluate (i.e. FLAG® -tagged recombinant protein purified from sample).
5. Immediately neutralize the eluate with 15 µL of Neutralization Buffer.
6. Regenerate the resins by repeating washing with 1X Wash Buffer for re-use.
7. For storage suspend the anti-FLAG® coupled to agarose beads in 1X Wash Buffer and add glycerol to 50%. A preservative may be added prior to storage at -20°C.

Post-purification analysis of the FLAG®-tagged recombinant protein

1. Prepare SDS-PAGE apparatus, gel and buffers for protein electrophoresis according to standard procedures. Include molecular weight markers for size comparison to purified FLAG®-tagged recombinant protein.
   Optional: include varying amounts of known control protein to create a standard curve for estimation of yield of your purified FLAG® -tagged recombinant protein.
2. Transfer aliquots containing a sufficient amount of neutralized purified FLAG®-tagged recombinant protein, unpurified lysate and the flow through solution (collected after sample loading) separately into clean microcentrifuge tubes with caps.
3. Add an equal volume of 2X SDS-PAGE Sample Buffer to each sample. Add reducing agent if desired to each sample. Boil samples prior to loading.
4. Load samples to gel. Connect electrodes. Separate by SDS-PAGE according to standard procedures.
5. Stain and destain gel for visualization. Document results and analyze the efficiency of purification by comparison to control proteins.

Notes

- The method given in these instructions is to be used as a guideline. Experienced users can make deviations from the outlined procedure. Note that the solutions have been optimized for the given method and alteration of the reagent concentrations, volumes, reaction times, or temperature will affect the overall performance of the kit. Generally, when modifying conditions experimentally, only alter one variable at a time.
- The anti-FLAG® coupled to agarose beads can withstand forces up to 5,000 x rpm without collapsing.

- The sample of cell lysate containing the FLAG® -tagged recombinant protein should be in phosphate buffered saline (PBS) or any other physiological buffer at or near neutral pH. The flow-through from sample loading is recommended to be saved and analyzed for comparison with starting material and eluted purified recombinant protein to ensure complete isolation of the fusion protein.
- Do not allow the anti-FLAG® coupled to agarose beads to remain in low pH elution buffer for longer than the stated time. Do not exceed more than 15 min of total contact time at low pH. Excessive exposure to low pH may inactivate the antibody preventing the regeneration and re-use of the agarose beads.
- Certain antigens and/or protein complexes may require a special lysis buffer composed of a different percentage of detergent. PBS is strongly recommended as a core buffer in any lysis buffer used in advanced applications of this kit. Alternative conditions must be determined empirically by the user. The anti-FLAG® antibody coupled to agarose beads is resistant to the following detergents: 5.0% TWEEN20, 5.0% TRITON X-100, 0.1% CHAPS and 0.2% digitonin as well as 1.0 M sodium chloride and 1.0 M urea.
- Never use the anti-FLAG® coupled to agarose beads with any concentration of sodium dodecyl sulfate (SDS), 2-mercaptoethanol (ß-ME), dithiothreitol (DTT), deoxycholate (DOC) or guanidine HCl. This is not a comprehensive list of interferences.
- The anti-FLAG® agarose bead resin can be reused up to three (3) times without loss of binding capacity when properly handled and stored. Spin columns also can be reused after sanitization and removal of nucleic acids and proteins using 0.1M NaOH.

Troubleshooting Guide

Issue	Cause	Solution
Low yield of eluted protein	Low binding efficiency, low elution efficiency or low protein expression	• Increase the amount of lysate/protein in the binding step and/or extend the incubation time to overnight at 4°C. • Use the provided FLAG® positive control lysate as a control in all experiments. • Repeat the elution step making certain that the agarose beads are well suspended in elution buffer prior to centrifugation. • Verify protein expression by SDS-PAGE gel or Western blotting prior to performing AntiDYKDDDDK (FLAG®) immunoprecipitation. We recommend using DYKDDDDK Tag (Anti-FLAG®) Antibody Peroxidase Conjugated (#200-303-383) for Western blotting.
Appearance of multiple bands by SDS-PAGE in eluted samples.	Washing step was not sufficient to eliminate non-specific binding	• Increase the number of washing steps after the sample is loaded onto the agarose beads. • Consider a more stringent washing buffer such as PBST. (See notes section)
	FLAG®-tagged recombinant protein expressed as multiple forms with varying molecular weights	Verify expression by Western blot. We recommend using DYKDDDDK Tag (Anti-FLAG®) Antibody Peroxidase Conjugated (#200-303-383).

References

Antibodies, A Laboratory Manual. Ed Harlow and David Lane, eds. Cold Spring Harbor Press, 1988.

Brizzard BL, Chubet RG, Vizard DL.(1994) Immunoaffinity purification of FLAG epitope-tagged bacterial alkaline phosphatase using a novel monoclonal antibody and peptide elution. Biotechniques 4:730-5.

Chiang CM, Roeder RG. (1993) Expression and purification of general transcription factors by FLAG epitope-tagging and peptide elution. Pept Res. 6(2):62-4.

Knappik A, Plückthun A. (1994) An improved affinity tag based on the FLAG peptide for the detection and purification of recombinant antibody fragments. Biotechniques 4:754-61.

Rockland products are for research use only and are not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Immunochemicals, Inc., P.O. BOX 5199, Limerick, Pennsylvania 19468, USA.

© 2025 Rockland Immunochemicals, Inc. All rights reserved.

FLAG® is a registered trademark of Merck KGaA.