Affinity-Purified ANTI-FLAG® M2 Antibody with High Specificity and Sensitivity

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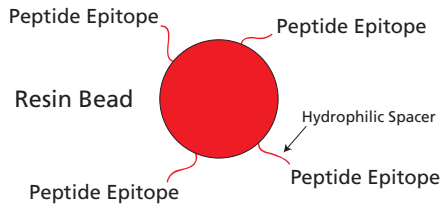
Abstract

The FLAG® system is a popular epitope tagging system for recombinant protein technology. The FLAG technology is comprised of a small 8 amino acid hydrophilic epitope tag (DYKDDDDK) and the corresponding monoclonal antibody(s). Monoclonal antibodies for research use are typically purified on either protein A or protein G affinity resin. We recently employed a proprietary, highly specific peptide affinity resin to purify an ANTI-FLAG M2 monoclonal antibody, thereby obtaining a final material that exhibits excellent specificity and high sensitivity. This affinity-purified ANTI-FLAG M2 antibody has been used to detect tagged fusion proteins in multiple expression systems. The affinity-purified ANTI-FLAG M2 antibody displays virtually exclusive selectivity for the target protein band in Western blot immunostaining in these expression systems. We also demonstrate the utility of affinity-purified ANTI-FLAG M2 antibody for indirect immunostaining of cells that are expressing epitope-tagged fusion proteins.

Introduction

Epitope tagging has become an essential tool for the detection and purification of expressed proteins. There are many different types of epitope tags, with the FLAG system being one of the most popular. The expression and purification of epitope-tagged fusion proteins has become routine in recombinant protein expression and proteomics applications. The small hydrophilic DYKDDDDK tag facilitates superior detection and purification of recombinant fusion proteins when using a highly specific and sensitive ANTI-FLAG antibody. The ANTI-FLAG M2 monoclonal antibody is useful for the detection, identification and capture of epitope-tagged fusion proteins by common immunological procedures such as Western blotting, immunoprecipitation, immunocytochemistry, and enzyme immunoassay (EIA). This poster will illustrate the superior performance attributes of this new affinity purified-antibody.



Methods

Preparation of Plant Extracts

Plant extract was prepared utilizing the Plant Total Protein Extraction Kit. Arabidopsis seedling tissue was ground to a fine powder in liquid nitrogen. The plant tissue was suspended in methanol and supplemented with protease inhibitor at –20 °C. The solution was centrifuged to pellet the proteins and washed twice with a methanol solution to remove any polyphenolics, tannins, or other interfering substances. The pellet was then washed with acetone and allowed to dry. Lastly, the protein was extracted using the Plant Protein Extraction Reagent Type 2, pelleted by centrifugation, and the soluble protein extract collected. The protein concentration of the extract was determined by Bradford assay.

Preparation of Bacterial Extracts

A starting culture was prepared by transferring one colony of *E. coli* strain JM109 to 5 mL of EZMix Terrific Broth (TB) in a 15 mL culture tube, and the tube was shaken overnight at 37 °C. One mL of the starter culture was transferred to 50 mL of fresh TB broth, and the cells were shaken for 8 hours at 37 °C. The cells were harvested by centrifugation and the supernatant removed. The cell pellet was re-suspended in Tris Buffered Saline (TBS), pH 8.0, sonicated to lyse the cells, and centrifuged. The protein concentration of the cell lysis solution was determined by Bradford assay.

Preparation of Mammalian Extracts

CHO cells from an actively growing culture were seeded at a density of 100,000 cells/ml into a T75 flask containing 30 mL of CHO medium, supplemented with 4 mM L-glutamine, and allowed to grow for 3 days. The cells were centrifuged and re-suspended in RIPA Buffer to a cell concentration of 2.0 x 10^7 cells/mL, supplemented with 1 mL of Protease Inhibitor per 10 mL of lysate. The protein concentration of the mammalian lysate was determined by Bradford assay.

Indirect Immunofluorescent Cytochemical Staining

Epitope-tagged p53 and mock transfected COS-7 cells were grown and transfected onto coverslips. The cells were fixed by incubation with PBS, pH 7.4, containing 4% paraformaldehyde and 4% sucrose for 15 minutes at room temperature. The fixed cells were washed twice with PBS for 5 minutes each, permeabilized by incubation with 0.25% TRITON™ X-100 in PBS for 5 minutes, and then washed twice with PBS for 5 minutes each. The cells were then blocked by incubation with 10% bovine serum albumin in PBS for 30 minutes at 37 °C. ANTI-FLAG M2 antibody, diluted 1/1,000 in 3% BSA/PBS, was incubated with the cells for 2 hours at 37 °C. The cells were washed three times with PBS for 5 minutes each and then incubated with anti-mouse FITC conjugated secondary antibody at a 1/1,000 dilution in 3% BSA/PBS for 45 minutes at 37 °C. Lastly, the cells were washed three times with PBS for 5 minutes each. The coverslips were mounted with the cells side down on glass slides using a small drop of polyvinyl alcohol with DABCO. Examination of the cells was performed by fluorescence microscopy at 492/520 nm. Alternatively, the cells were also stained with DAPI at a dilution of 1/5,000 and examined by fluorescence microscopy at 365/460 nm.

Western Blotting

Based on the results of the Bradford assay, 10 µg of protein from either bacterial, mammalian, or plant extract, supplemented with or without 10 ng of Amino-terminal FLAG-BAP Fusion Protein, were separated on a 4–20% SDS-PAGE gel.

The proteins were transferred from the gel to a nitrocellulose membrane. The membrane was blocked with Tris buffered saline (TBS) in 3% nonfat dry milk for 30 minutes. The blot was then incubated with 1 µg/mL of the ANTI-FLAG M2 monoclonal antibody in TBS-milk at room temperature with shaking for 30 minutes. Then the blot was incubated with Anti-Mouse IgG Peroxidase at a 1:30,000 dilution in TBS-milk for 30 minutes and washed three times for a total of 15 minutes in TBS-Tween® 20. Finally, the blot was developed for 5 minutes with an HRP chemiluminescent substrate, CPS-1.

Materials

- Monoclonal ANTI-FLAG M2, Affinity Purified (Product Code F 1804)
- DAPI (Product Code D 8417)
- Polyvinyl alcohol mounting medium with DABCO, anti-fading (Product Code 10981)
- Chemiluminescent Peroxidase Substrate-1 (Product Code CPS-1-120)
- Anti-Mouse IgG Peroxidase (Product Code A 9044)
- Plant Total Protein Extraction Kit (Product Code PE 0230)
- Protease Inhibitor (Product Code P 8340)
- CHO Medium (Product Code C 5467)
- RIPA Buffer (Product Code R 0278)
- Terrific Broth, Modified EZMix Powder (Product Code T 9179)
- Amino-terminal FLAG-BAP Fusion Protein (Product Code P 7582)
- Anti-Mouse IgG-FITC Antibody (Product Code F 9137)
- Albumin Bovine Serum (Product Code A 9647)
- Triton™ X-100 (Product Code T 9284)
- EZBlue™Gel Staining Reagent (Product Code G 1041)

Results

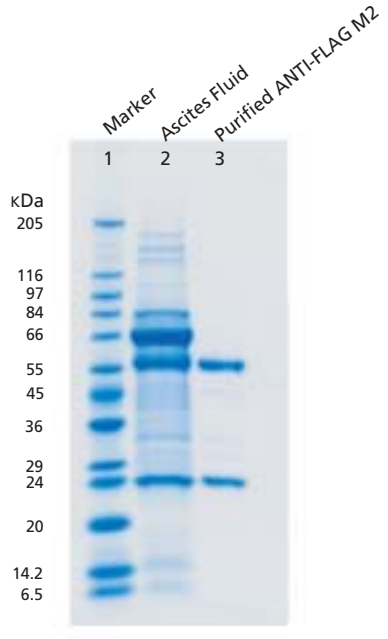


Figure 1. Demonstration of Purity
SDS-PAGE analysis of purified ANTI-FLAG M2 monoclonal antibody from mouse ascites fluid. **Lane 2** contains mouse ascites fluid containing 2.5 µg of ANTI-FLAG M2. **Lane 3** contains 2.5 µg of purified ANTI-FLAG M2 antibody. Proteins separated by SDS-PAGE and stained with EZBlue™Gel Staining Reagent.

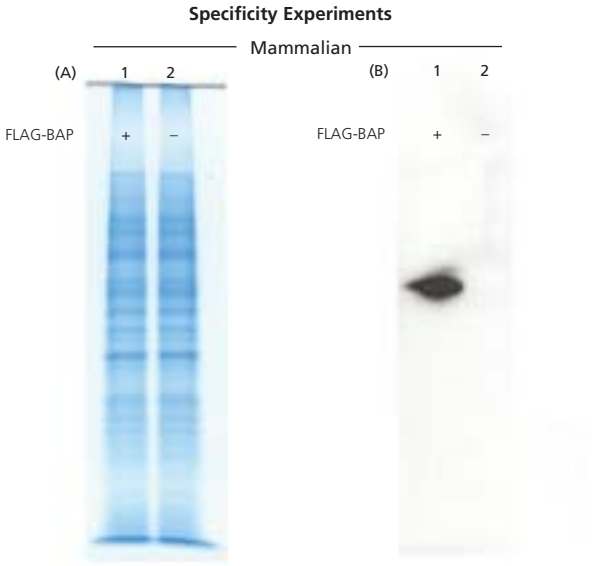


Figure 2. Demonstration of Selectivity in a Mammalian System

A) Proteins separated by SDS-PAGE and stained with EZBlue™Gel Staining Reagent. **Lane 1** contains 10 ng of spiked FLAG-BAP in 10 µg of CHO mammalian lysate. **Lane 2** contains only 10 µg of CHO lysate.

B) Western blot utilizing the new ANTI-FLAG M2 to detect the spiked FLAG protein in CHO lysate. **Lane 1** contains 10 ng of spiked FLAG-BAP in 10 µg of CHO mammalian lysate. **Lane 2** contains only 10 µg of CHO lysate. Note the excellent specificity of detection.

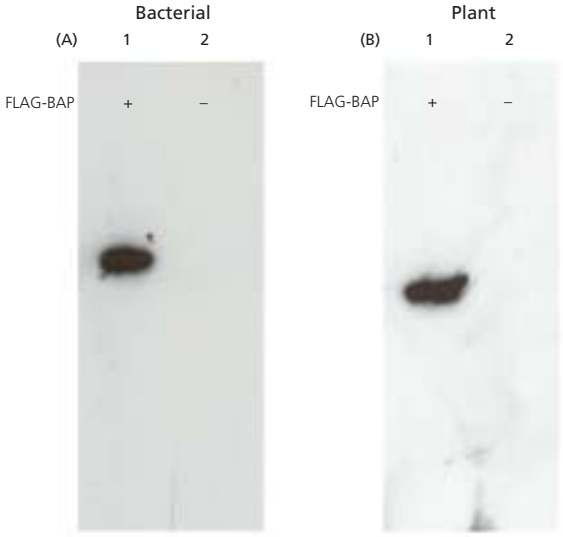


Figure 3. Demonstration of Selectivity in a Bacterial and Plant System

A) Western blot utilizing the new ANTI-FLAG M2 to detect the spiked FLAG protein in *E. coli* lysate. **Lane 1** contains 10 ng of spiked FLAG-BAP in 10 µg of *E. coli* bacterial lysate. **Lane 2** contains only 10 µg of *E. coli* bacterial lysate. Note the excellent specificity of detection.

B) Western blot utilizing the new ANTI-FLAG M2 to detect the spiked FLAG protein in Arabidopsis seedling lysate. **Lane 1** contains 10 ng of spiked FLAG-BAP in 10 µg of Arabidopsis seedling lysate. **Lane 2** contains only 10 µg of Arabidopsis seedling lysate. Note the excellent specificity of detection.

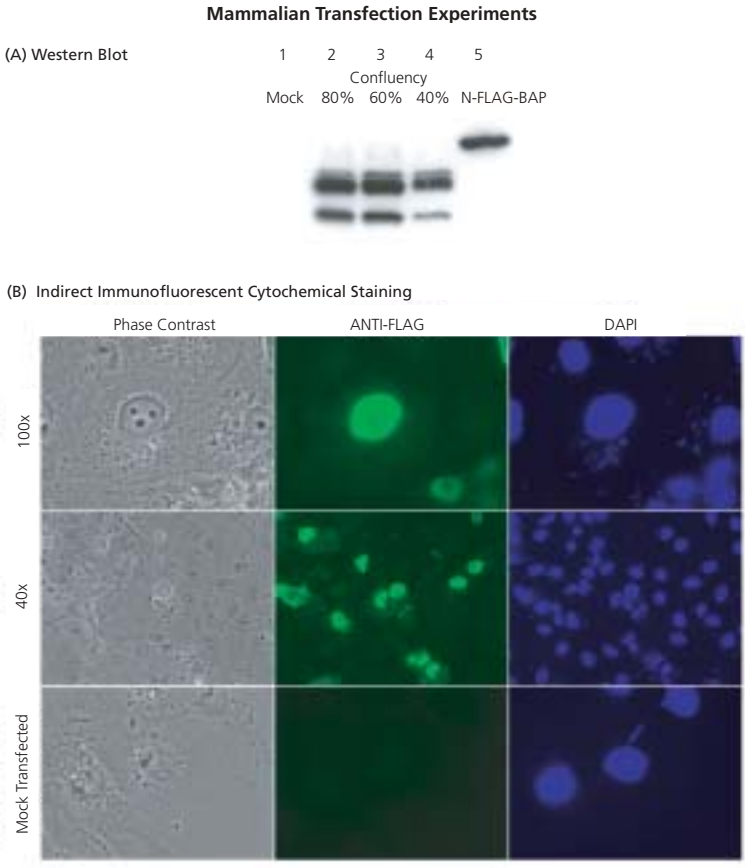


Figure 4. Cytochemical Staining

A) Western blot of mammalian cells expressing epitope-tagged p53 (Δ1-71). **Lane 1** contains mock transfected at 80% confluency at time of transfection. **Lane 2** through **Lane 4** contain epitope-tagged p53 (Δ1-71) transfected cells at 80%, 60%, and 40% confluency. **Lane 5** contains 9 ng of control N-FLAG-BAP.

B) Epitope-tagged p53 and mock transfected COS-7 cells were permeabilized, fixed, blocked, and incubated with the new ANTI-FLAG M2. A secondary antibody conjugated with FITC was used to detect the primary antibody. The cells were also stained with DAPI.

Conclusion

The FLAG system is a popular epitope tagging system for recombinant protein technology. We have employed an affinity resin to purify an ANTI-FLAG M2 monoclonal antibody exhibiting excellent specificity and high sensitivity. This affinity-purified ANTI-FLAG M2 antibody has been utilized to detect tagged fusion proteins in multiple expression systems, displaying virtually exclusive selectivity for the target protein band in Western blot immunostaining. We also demonstrated the utility of ANTI-FLAG M2 monoclonal antibody for indirect immunostaining of cells that are expressing epitope-tagged fusion proteins. The above experiments illustrate the superior performance attributes of this new affinity-purified antibody.

References

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