Superior Performance of a New Affinity Purified ANTI-FLAG® M2 Antibody

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- **Superior specificity:**
  – Detects a single band of protein on a Western blot by chemiluminescent probing
- **High sensitivity:**
  – Detects down to 1 ng of FLAG-BAP™ on a Western Blot
- **Extremely stable:**
  – Stable for greater than 3 years

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 FLAG technology consists of a small 8 amino acid hydrophilic epitope tag (DYKDDDDK) and the corresponding monoclonal antibody(s). 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). We recently employed a proprietary, highly specific peptide affinity resin to purify the ANTI-FLAG M2 monoclonal antibody, thereby obtaining a final material that exhibits excellent specificity and high sensitivity. This article illustrates the superior performance attributes of this new affinity purified antibody.

Monoclonal antibodies for research use are typically purified on either Protein A or Protein G affinity resin. This purification scheme is not optimal, in that any endogenous antibodies present, or damaged, non-functional monoclonal antibodies, are co-purified. In order to obtain a highly specific, ultra pure ANTI-FLAG antibody, we employed a unique FLAG peptide resin to specifically purify functional ANTI-FLAG M2 antibody. In order to test the selectivity of the new ANTI-FLAG M2, Affinity Purified antibody (Product Code F1804), Western blot immunostaining was utilized (Figure 1). Purified FLAG-BAP, an N-terminal FLAG fusion protein of *E. coli* bacterial alkaline phosphatase (49.3 kDa), was spiked into mammalian CHO lysate. SDS-electrophoresis was performed and the separated proteins transferred to a nitrocellulose membrane. The new ANTI-FLAG M2 was used to probe for the FLAG-BAP fusion protein, followed by a rabbit anti-mouse HRP conjugate (Product Code A9044). Chemiluminescent peroxidase substrate (CPS-1) was used for detection of the peroxidase labeled secondary antibody.

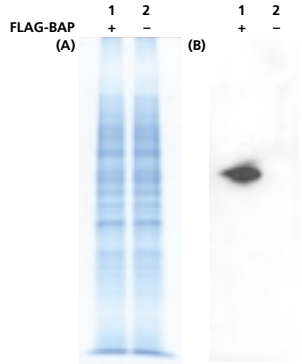

Figure 1. Demonstration of selectivity. (A) *Proteins separated by SDS-PAGE and stained with EZBlue™ Gel Staining Reagent. Lane 1 contains 10 ng of spiked FLAG-BAP in 10 µg of CHO mammalian lysate. Lane 2 contains only 10 µg of CHO lysate;* ***(B)*** *Western blot utilizing the new ANTI-FLAG M2 to detect 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.*

A Western blot was performed to demonstrate that the ANTI-FLAG M2, Affinity Purified antibody displays exquisite specificity for the epitope-tagged fusion protein. The accompanying 1D gel shows that many proteins are present in both the spiked and pure CHO lysate. The blot shows that only the epitope-tagged fusion protein is detected by the new ANTI-FLAG M2.

Western Blot immunostaining was also used to test the sensitivity of the ANTI-FLAG M2, Affinity Purified antibody (Figure 2). Purified FLAG-BAP was spiked into CHO lysate at concentrations of 4, 2, 1, and 0.5 ng. The new ANTI-FLAG was used at a dilution of 1/1,000, followed by a rabbit, anti-mouse HRP conjugate (Product Code A9044) diluted 1/30,000. Chemiluminescent peroxidase substrate was used for the detection of the peroxidase labeled secondary antibody. The Western blot shows that the epitope-tagged fusion protein, FLAG-BAP, may be detected down to a concentration of 1 ng.

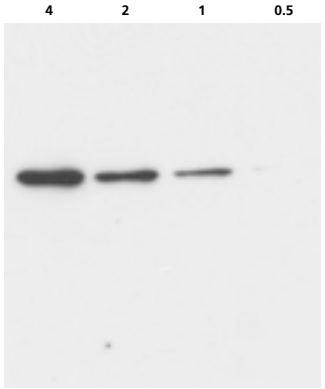

Figure 2. Demonstration of sensitivity. *Western blot utilizing the new ANTI-FLAG M2 to detect varying amounts of spiked FLAG protein in CHO lysate. All lanes contain 10 µg of CHO lysate spiked with 4, 2, 1, or 0.5 ng of FLAG-BAP.*

Cytochemical staining of cells expressing an epitope-tagged fusion protein was performed in order to test the new ANTI-FLAG M2 in a system employing indirect immunofluorescence (Figure 3).

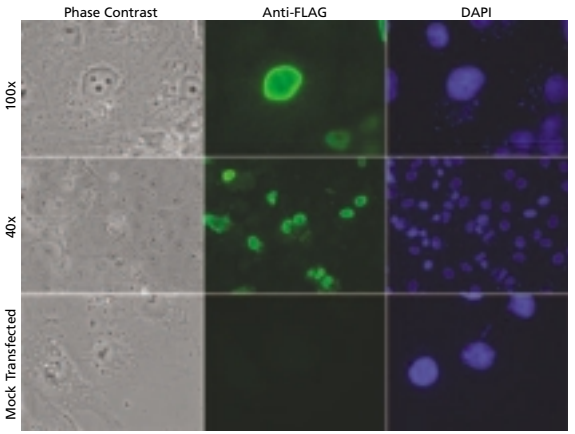

Figure 3. Cytochemical staining. *FLAG-p53 and mock transfected COS-7 cells were permeabilized, fixed, blocked, and incubated with the new ANTI-FLAG M2. A secondary antibody conjugated to FITC was used to detect the primary antibody. The cells were also stained with DAPI.*

A vector expressing the fusion protein FLAG-p53 was transfected into COS-7 mammalian cells. The cells were applied to microscope slides, permeabilized, fixed, blocked, and incubated with the new ANTI-FLAG M2. A FITC conjugated secondary anti-mouse antibody (Product Code F9137) was used to detect the primary antibody. Cells were also stained with DAPI, a nucleus staining reagent. The p53 protein contains a nuclear localization signal near the carboxyl terminus end of the protein, and therefore should be limited to the nucleus.

The slides show that no signal is detected in the mock transfected cells, demonstrating the specificity of the ANTI-FLAG M2, Affinity Purified antibody. The new ANTI-FLAG M2 did not bind to proteins lacking the epitope tag, preventing non-specific background FITC generated fluorescence. In the transfected cells, the DAPI slide stains the nuclei of all cells, while the FLAG slide displays only those cells expressing the epitope-tagged fusion protein.

Ordering Information

Product	Description	Unit
F1804	Monoclonal ANTI-FLAG M2 Antibody, Affinity Purified	0.2 mg 1 mg 5 mg