

# CLONING AND EXPRESSION

## FLAG® and 3xFLAG® Overview

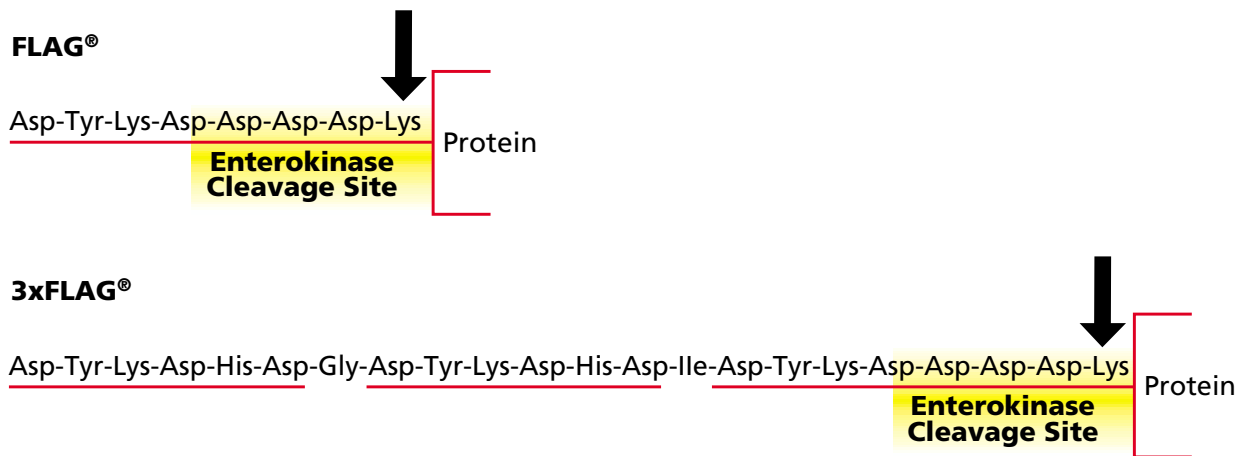
### A Proven System for Detection and Purification of Proteins

The FLAG Expression System is an established way to express, purify and detect recombinant fusion proteins. FLAG and 3xFLAG have proven utility in numerous applications such as Western blotting, immunocytochemistry, immunoprecipitation, flow cytometry, protein purification, and in the study of protein-protein interactions, cell ultrastructure and protein localization. These small hydrophilic tags facilitate superior detection and purification of recombinant fusion proteins when using our highly specific and sensitive ANTI-FLAG® antibodies.

### Ideal Epitope Tags: Small, Hydrophilic and Cleavable

The FLAG expression system utilizes a short, hydrophilic 8-amino acid peptide that is fused to the recombinant protein of interest. Because of its hydrophilic nature, the FLAG peptide is likely to be located on the surface of the fusion protein. As a result, the FLAG peptide is easily accessible for cleavage by enterokinase (Ek) and for detection with antibodies. In addition, because of the small size of the FLAG peptide tag, it is not likely to obscure other epitopes, domains, or alter function, secretion, or transport of the fusion protein.

The 3xFLAG system is an improvement upon the original system by fusing 3 tandem FLAG epitopes (22 amino acids). Detection of fusion proteins containing 3xFLAG is enhanced up to 200 times more than any other system. Like the original FLAG tag, 3xFLAG is hydrophilic, contains an Ek cleavage site, and is relatively small. Therefore, the risk of altering protein function, blocking other epitopes or decreasing solubility is minimized.

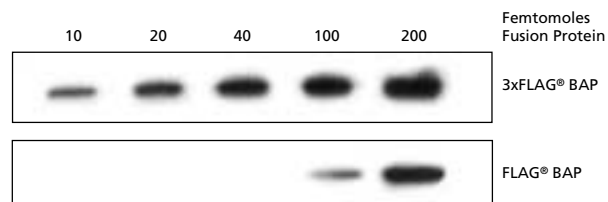


**Figure 1. FLAG and 3xFLAG Amino Acid Sequences**

Removal of N-terminal FLAG (8 amino acids) and 3xFLAG (22 amino acids) tags is possible using enterokinase, which cleaves following the Asp-Asp-Asp-Lys amino acid sequence at the c-terminal end of the tags.

### Highly Sensitive

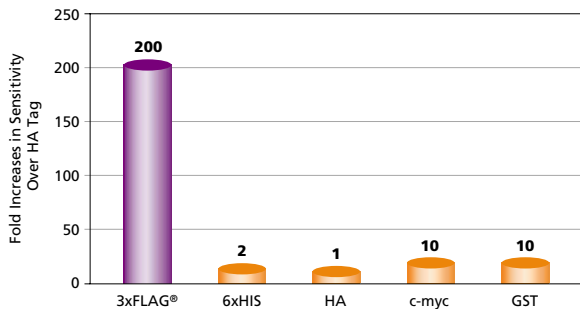
- FLAG; detect ~ 100 femtomoles
- 3xFLAG; Detect ~ 10 femtomoles



**Figure 2. Western Blot of Original FLAG® Versus 3xFLAG®**

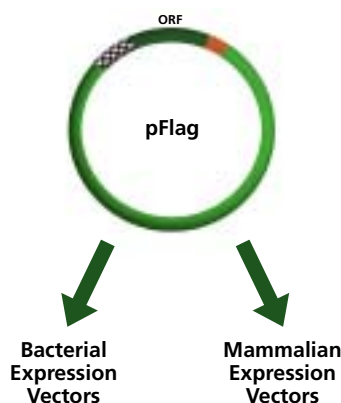
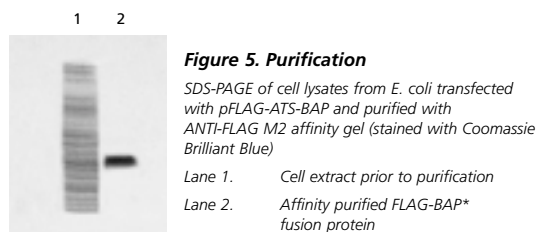
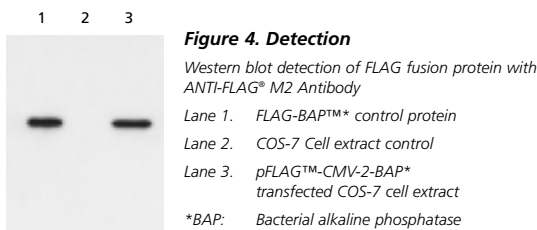
Western blot of purified 3xFLAG® bacterial alkaline phosphatase and FLAG® bacterial alkaline phosphatase transferred onto a nitrocellulose membrane. Detection was performed with ANTI-FLAG® M2 monoclonal primary antibody, anti-mouse-HRP secondary antibody, and ECL™ chemiluminescent substrate.

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**Figure 3. Comparative Sensitivity of 3xFLAG®**

Each protein fusion tag was cloned separately on the C terminal of GST. The purified proteins were diluted from 1 µg to 0.01 ng and analyzed. The limit of detection of each tag was determined by Western blot analysis using the recommended dilutions of each respective primary antibody and secondary anti-mouse IgG-HRP conjugate. Blot was developed using ECL.



## FLAG® and 3xFLAG® Overview

### 3xFLAG

- Ultrasensitive; 20-200x more sensitive than any other system
- Detect ~ 10 femtomoles
- Ideal for cases of low-level expression in mammalian cells
- 3 tandem repeats of the FLAG sequence
- Enhanced detection for immunoprecipitation, Western blots and immunocytochemistry

### Highly Specific Antibodies

- FLAG and 3xFLAG sequences include the binding sites for several highly specific ANTI-FLAG monoclonal (M1, M2, M5) and polyclonal antibodies and conjugates, each with different recognition and binding characteristics
- ANTI-FLAG antibodies exhibit little or no cross-reactivity in most mammalian and bacterial cell lysates

### One Step Purification

- Single band purity with only one chromatography step
- Non-denaturing purification
- Simple, competitive elution using FLAG and 3xFLAG synthetic peptides
- Purification formats include affinity gels and 96-well plates

### Vectors for Bacterial and Mammalian Systems

- Cytoplasmic expression or secretion
- Bacterial vectors with T7 or tac promoters
- 3xFLAG, as well as FLAG, offered for mammalian systems
- Options for dual-tagged fusion proteins
- N-terminal FLAG and 3xFLAG vectors provide an E<sub>k</sub> cleavage site for removal of the fusion tag
- New BICEP™ line of vectors for bicistronic expression in mammalian cells; offer high performance stable expression of FLAG fusions or multi-gene expression possibilities