Invitrogen™
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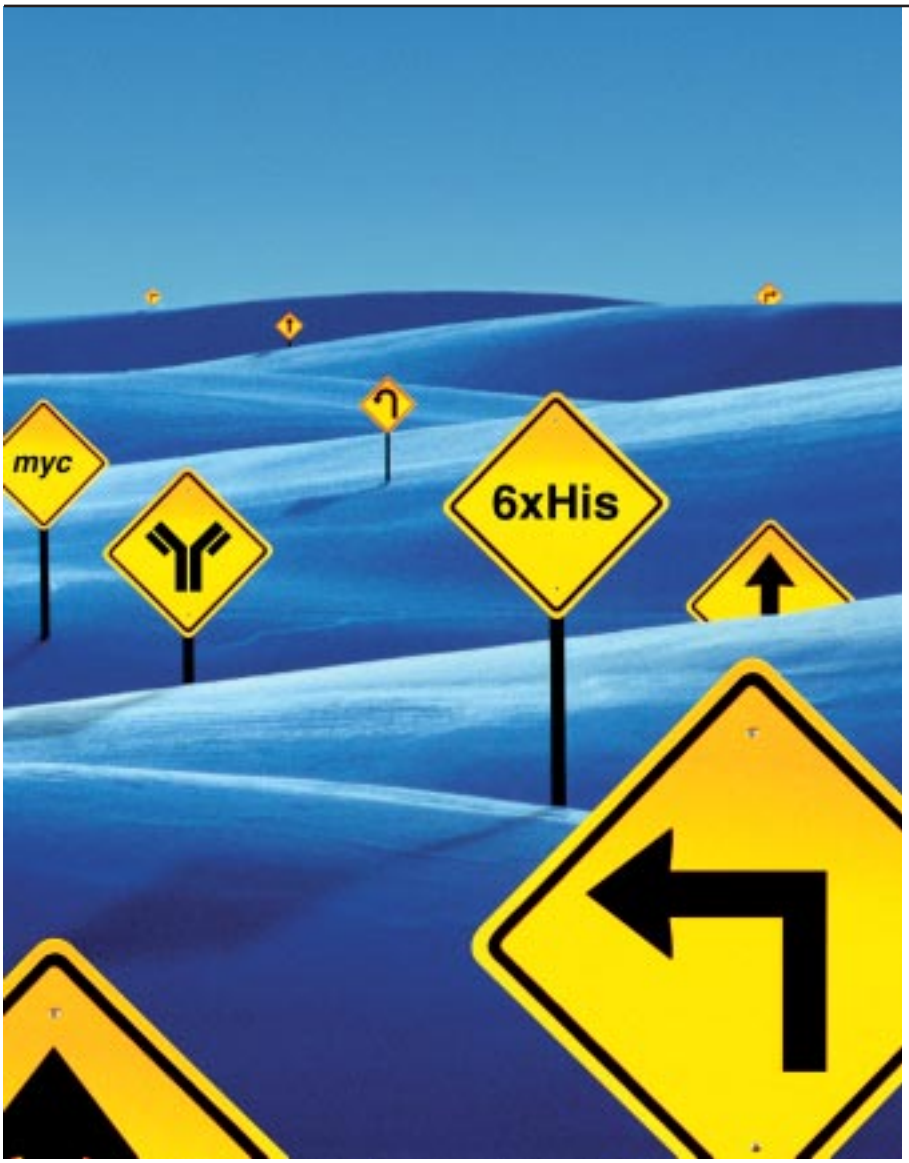
Simple, rapid detection of fusion proteins



Monoclonal antibodies for detection of epitope tags give you:

- Convenient, rapid detection of fusion proteins
- Low backgrounds, high specificity
- Flexibility of a variety of conjugates

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High-quality antibodies for clear results

Antibodies against epitope tags are an efficient, convenient and rapid method for detecting recombinant protein expression. Invitrogen offers highly specific monoclonal antibodies against some of the most widely used epitope tags in both unconjugated and conjugated formats. There's no need to spend time and effort raising your own antibody, or setting up antibody production capabilities. Ensure clean immunodetection results and cut detection time with Invitrogen's high-quality monoclonals.

Simple and effective protein detection

Use the powerful strategy of epitope tagging for detecting, analyzing, and purifying proteins. Using recombinant DNA techniques, a small peptide is transferred to the protein of interest to produce a fusion protein. Antibodies engineered against the specific peptide tag, or epitope, are then used to detect the fusion protein. This simple and effective process is one of the most widely used techniques for both *in vivo* and *in vitro* protein studies. A variety of antibodies to epitopes used in Invitrogen's expression systems are available, including antibodies against the V5, c-*myc* and Xpress™ epitopes, and both C- and N- terminal polyhistidine (6xHis) tags (Table 1).

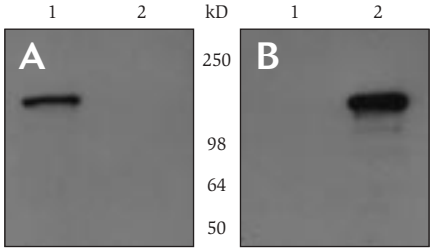
Table 1 - Antibodies available

Antibody	Recognition sequence
Anti-V5	-Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr-
Anti-*myc*	-Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu-
Anti-His(C-term)[22]	-His-His-His-His-His-His-COOH
Anti-HisG[22]	-His-His-His-His-His-His-Gly-
Anti-Xpress™	-Asp-Leu-Tyr-Asp-Asp-Asp-Asp-Lys-

Detect only the protein you want

Highly specific, these antibodies recognize only their respective epitopes, so you'll see low backgrounds and accurate, specific signals. Their unmatched specificity and consistency also make them ideal for use in western blot, immunofluorescence, ELISA and immunoprecipitation (Figures 1 and 2).

Figure 1 - Detection with Anti-*myc* and Anti-V5 Antibodies



Lysates from 293 cells expressing *lacZ* from the pcDNA3.1/ *myc*-His® or pcDNA3.1/V5-His® vector were separated (2 x 10^5 cells/lane) by SDS-PAGE. The gels were blotted and probed with either the Anti-*myc* (Panel A) or Anti-V5 (Panel B) Antibodies (1:5000, 0.2 µg/ml). Epitope tagged proteins were detected with an HRP-conjugated secondary antibody and chemiluminescence substrate.

Lane 1 (A & B): Expression from pcDNA3.1/*myc*-His®/*lacZ*
Lane 2 (A & B): Expression from pcDNA3.1/V5-His®/*lacZ*

A choice of conjugates – choose the best format

Most of Invitrogen's epitope antibodies are available in both unconjugated and conjugated formats. Epitope tag detection with an unconjugated antibody requires the use of a secondary antibody that carries a reporter for detection. Unconjugated antibodies let you choose the detection method which best suits your needs. They are also useful for the detection of weak signals, since a secondary antibody can amplify the signal.

Conjugated antibodies (Table 2) offer the benefits of time savings and clearer signals with lower backgrounds. Since the antibody already has a detection label, you don't need to use a labeled secondary antibody or to optimize the detection procedure. Conjugated antibodies eliminate the cross-reactivity and non-specific signals that can be associated with secondary antibodies. Most of Invitrogen's antibodies are available conjugated to one of three different reporters:

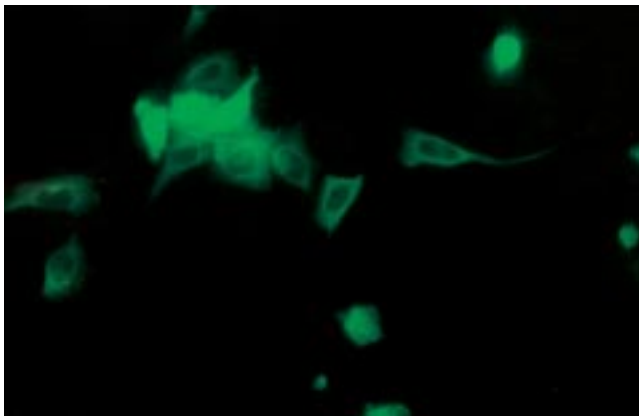
- **Horseradish peroxidase (HRP)**
- **Alkaline phosphatase (AP)**
- **Fluorescein isothiocyanate (FITC)**

- **Horseradish peroxidase (HRP)**-conjugated antibodies are most often used together with a chemiluminescent substrate for fast and sensitive detection of signals on film or with phosphorimagers. Signals are usually detected in minutes. Also commonly used with chromogenic substrates used in immunohistochemistry.

- **Alkaline phosphatase (AP)**-conjugated antibodies are frequently used with chromogenic substrates to generate western blotting results on a membrane, eliminating the time-consuming use of film and film processing. Detect signal directly on your benchtop with a chromogenic substrate. Detection time is extended, as well: signal generated by AP creates a permanent signal on your blot, compared to minutes with HRP.

- **Fluorescein isothiocyanate (FITC)**-conjugated antibodies provide high sensitivity and resolution in fluorescent detection. Fluorescent labels, like FITC, are widely used in immunofluorescence, flow cytometry (FACS), and protein localization studies.

Figure 2 - Immunofluorescence with the Anti-V5-FITC Antibody



1 x 10^5 CHO cells at 50% confluence were transfected with pcDNA3.1/V5-His*/β-tubulin (GeneStorm® human ORF, H-AF070600M). Thirty hours post-transfection, cells were washed with PBS and fixed with 100% methanol. After blocking with 10% fetal bovine serum in PBS, cells were incubated for one hour with Anti-V5-FITC Antibody at a 1:500 dilution. Cells were viewed with an Olympus® fluorescence microscope using a FITC filter.

A choice of conjugates – choose the best format

Table 2 - Conjugated antibodies available from Invitrogen

Antibody	Class	Recognition sequence	Available conjugates	Application
Anti-Xpress™	IgG_1	-Asp-Leu-Tyr-Asp-Asp-Asp-Asp-Lys-	HRP FITC	western blot ELISA Immunoprecipitation Immunostaining
Anti-V5	IgG_{2a}	-Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr-	HRP AP FITC	western blot ELISA immunoprecipitation Immunostaining
Anti-*myc*	IgG_1	-Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu-	HRP AP FITC	western blot ELISA immunoprecipitation immunostaining
Anti-His(C-term)	IgG_{2b}	-His-His-His-His-His-His-COOH	HRP AP FITC	western blot ELISA Immunostaining
Anti-HisG	IgG_{2a}	-His-His-His-His-His-His-Gly-	HRP AP	western blot ELISA immunoprecipitation
Anti-Thio™29	IgG_{1k}			western blot ELISA

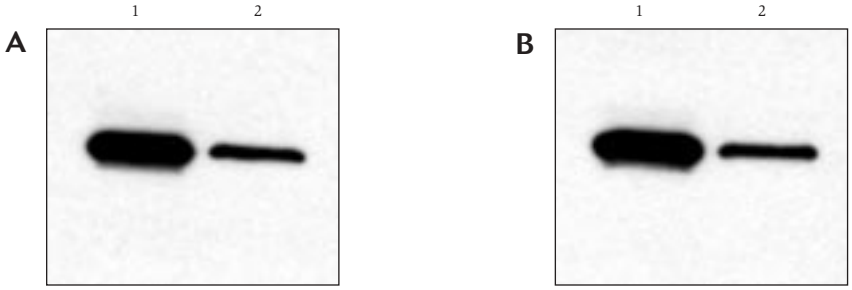
Quality guaranteed

We extensively test each antibody to ensure the highest quality. Every lot is affinity purified and functionally tested in western blot and ELISA to ensure specific binding and low background. Sufficient antibody is provided for 25 western blots.

Ensured results for western blots

The Positope™ Control Protein55 gives you the control you need for reliable western blots. It's designed to be used as a positive control in western blotting for a variety of antibodies (Figure 3). The control protein is a 53 kD purified (>95%) recombinant protein that contains seven epitope tags (Table 3). It's quality tested by western blot against each of the epitopes. Use Positope™ as a positive control to assess the performance of your western blots, no matter which epitope you use.

Figure 3 - Positope™ Control Protein detected with the Anti-HisG and Anti-GFP Antibodies



100 ng (lane 1) and 25 ng (lane 2) of the Positope™ Control Protein were separated by SDS-PAGE and transferred to seven nitrocellulose membranes. Membranes were individually probed with 2 µl of either Anti-Thio™, Anti-HisG (Panel A), Anti-Xpress™, Anti-GFP (Panel B), Anti-*myc*, Anti-V5, or Anti-His(C-term) Antibody in 20 ml TPBS + 2% NFM for 2 hours. The membranes were washed three times and then probed with 20 µl of HRP-conjugated secondary antibody in 20 ml TPBS + 2% NFM for 2 hours. Membranes were washed four times and developed with chemiluminescence reagents.

Table 3 - The epitopes in Positope™ Control Protein*

Tag	Description
Xpress™	-DLYDDDDK-
c-myc	-EQKLISEEDL-
V5	-GKPIPNPLLGLDST-
His(C-term)	-HHHHHH-COOH-
HisG	-HHHHHHG-
Thioredoxin	His-Patch Thioredoxin
GFP	Green Fluorescent Protein

* Recommended loading amount for western blot: 100 ng per lane

Enjoy the convenience

Reduce the amount of time you spend detecting fusion proteins. Get high-specificity, low-background results with Invitrogen's monoclonal antibodies. Order yours today.

Product	Class	Quantity	Cat. no.
Anti-V5 Antibody	IgG_{2a}	50 µl	R960-25
Anti-V5 Antibody	IgG_{2a}	500 µl	R960-CUS
Anti-V5-HRP Antibody	IgG_{2a}	50 µl	R961-25
Anti-V5-AP Antibody	IgG_{2a}	125 µl	R962-25
Anti-V5-FITC Antibody	IgG_{2a}	50 µl	R963-25
Anti-*myc* Antibody	IgG_1	50 µl	R950-25
Anti-*myc* Antibody	IgG_1	500 µl	R950-CUS
Anti-*myc*-HRP Antibody	IgG_1	50 µl	R951-25
Anti-*myc*-AP Antibody	IgG_1	125 µl	R952-25
Anti-*myc*-FITC Antibody	IgG_1	50 µl	R953-25
Anti-Xpress™ Antibody	IgG_1	50 µl	R910-25
Anti-Xpress™ Antibody	IgG_1	500 µl	R910-CUS
Anti-Xpress™-HRP Antibody	IgG_1	50 µl	R911-25
Anti-Xpress™-FITC Antibody	IgG_1	50 µl	R913-25
Anti-His(C-term) Antibody	IgG_{2b}	50 µl	R930-25
Anti-His(C-term) Antibody	IgG_{2b}	500 µl	R930-CUS
Anti-His(C-term)-HRP Antibody	IgG_{2b}	50 µl	R931-25
Anti-His(C-term)-AP Antibody	IgG_{2b}	125 µl	R932-25
Anti-His(C-term)-FITC Antibody	IgG_{2b}	50 µl	R933-25
Anti-HisG Antibody	IgG_{2a}	50 µl	R940-25
Anti-HisG-HRP Antibody	IgG_{2a}	50 µl	R941-25
Anti-HisG-AP Antibody	IgG_{2a}	125 µl	R942-25
Positope™ Control Protein		5µg	R900-50

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