Positope™

Catalog no. R900-50

General Information

Introduction

Positope™ is a recombinant protein specifically engineered to contain seven different tags for detection with seven different antibodies. Positope™ protein is intended for use as a positive control for antibody function in western blot experiments.

Shipping/Storage

The Positope™ protein is shipped at room temperature. Upon receipt, please store at +4°C. The protein is stable for 6 months at +4°C.

Contents

Positope™ recombinant protein is supplied in a total volume of 200 µl at a concentration of 25 ng/µl in reducing SDS-PAGE sample buffer (63 mM Tris-HCl, 10% glycerol, 2% SDS, 0.0025% Bromophenol Blue, 50 mM β-mercaptoethanol).

Product Qualification

Each lot of Positope™ must be purified to > 95% purity from an *E. coli* lysate. In addition, each primary antibody listed below will detect 250 ng of Positope™ on a western blot using HRP-conjugated secondary antibody and chemiluminescence reagents. A strong signal is observed after a 1 minute exposure.

Description

Positope™ is a 53 kDa recombinant protein created using the gene for green fluorescent protein (GFP) and engineered to contain six additional epitopes as shown in the figure. It is expressed from pBAD/Thio-TOPO® in TOP10 cells using arabinose as an inducer. The table describes the actual epitope if known. Amino acid sequence is available by contacting Technical Service (page 3).

Note: HRP conjugates of Invitrogen antibodies, i.e. Anti-*myc*-HRP, Anti-V5-HRP, Anti-Xpress™-HRP, Anti-HisG-HRP, and Anti-His(C-term)-HRP, also detect Positope™.

Tag	Epitope	Reference
Thioredoxin	Unknown	(Dickason *et al.*, 1995)
HisG	-HHHHHHG-	(Robson *et al.*, 1995)
Xpress™	-DLYDDDDK-	(Kroll *et al.*, 1993)
GFP	Many	Invitrogen
c-myc	-EQKLISEEDL-	(Evan *et al.*, 1985)
V5	-GKPIPNPLLGLDST-	(Southern *et al.*, 1991)
His(C-term)	-HHHHHH-COOH	(Lindner *et al.*, 1997)

Using Positope™

Please note that the Positope™ control protein is provided in reducing SDS-PAGE sample buffer and is **not** suitable for use in immunoassays (i.e. ELISA).

Western Analysis

In experiments performed at Invitrogen, we tested various amounts of Positope™ to obtain a strong signal in a western blot. Results are shown in the table below. Conditions were:

Primary antibody: 2 µl primary antibody in 20 ml buffer (1:10,000)
HRP-conjugated secondary antibody (goat anti-mouse-HRP or goat anti-rabbit-HRP): 1:10,000 dilution
Detection: Chemiluminescence reagents (ECL; Amersham)
Exposure: 1 minute

Primary Antibody	Amount of Positope™
Anti-Thio	100 ng
Anti-HisG	100 ng
Anti-Xpress™	250 ng
Anti-GFP (polyclonal)	100 ng
Anti-*Myc*	250 ng
Anti-V5	100 ng
Anti-His(C-term)	250 ng

Recommended Use

For Western blot analysis, we recommend using 250 ng (10 µl) of Positope™ per lane. Lower amounts of Positope™ may be used, but the strength of the signal may be affected.

Positope™ recombinant protein is supplied in reducing SDS-PAGE sample buffer. We recommend that you perform the following steps before use:

1. Transfer the appropriate amount of Positope™ that you will load onto the gel (e.g. 10 µl) to a separate microcentrifuge tube.
2. Treat the Positope™ in the same manner as your other samples prior to loading your gel. We generally boil the sample for five minutes.

Note

Increasing the amount of Positope™ loaded, using too much antibody, or increasing the detection/exposure time may lead to the detection of proteolytic breakdown products of Positope™, especially if you are using the Anti-V5 Antibody.

For More Information

For more information on your specific antibody or for procedures to perform western blot analysis, please refer to the particular manual for your antibody.

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
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- Request catalog and product literature

Once connected to the Internet, launch your web browser (Netscape 3.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).

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