

Positope™

Cat. No. R900-50

Store at 4°C

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 and 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.

Intended Use

For research use only. **Not intended for human or animal diagnostic or therapeutic uses.**

Contents

The 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).

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 Support (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 <i>et al.</i> , 1995)
HisG	-HHHHHHG-	(Robson <i>et al.</i> , 1995)
Xpress™	-DLYDDDDK-	(Kroll <i>et al.</i> , 1993)
GFP	Many	Invitrogen
<i>c-myc</i>	-EQKLISEEDL-	(Evan <i>et al.</i> , 1985)
V5	-GKPIPPLLGLDST-	(Southern <i>et al.</i> , 1991)
His(C-term)	-HHHHHHH-COOH	(Lindner <i>et al.</i> , 1997)

Using Positope™

Caution

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

Technical Support

Web Resources

Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

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

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SDS

Safety Data Sheets (SDSs) are available at www.invitrogen.com/sds.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

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