SANTA CRUZ BIOTECHNOLOGY, INC.

VP16 (14-5): sc-7546

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

The GAL4 protein of *Saccharomyces cerevisiae* is one of the most thoroughly characterized transcriptional activators. Since the N-terminal 147 amino acid residues of GAL4 are sufficient to mediate specific and strong binding to DNA, but are incapable of efficient transcriptional activation, this protein fragment has frequently been used to confer specific DNA binding in experiments examining transcriptional activation functions of heterologous proteins. This approach is facilitated by the finding that higher eukaryotes lack endogenous proteins that enhance transcription from the consensus GAL4-binding site. Fusions between GAL4 (aa 1-147) and activating domains from a variety of transcriptional regulatory proteins can activate transcription in yeast, plant, insects and mammalian cells. A unique "two-hybrid" system has been developed using GAL4 fusions in yeast to identify specific protein-protein interactions. Another "two-hybrid" system utilizes the DNA binding domain of the *E. coli* protein Lex A and the transactivity domain of the HSV protein VP16.

SOURCE

VP16 (14-5) is a mouse monoclonal antibody epitope corresponding to amino acids 410-456 at the N-terminal transcription activation domain of VP16.

PRODUCT

Each vial contains 200 µg IgG_{2a} kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

VP16 (14-5) is available conjugated to agarose (sc-7546 AC), 500 µg/0.25 ml agarose in 1 ml, for IP; to HRP (sc-7546 HRP), 200 µg/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-7546 PE), fluorescein (sc-7546 FITC), Alexa Fluor® 488 (sc-7546 AF488), Alexa Fluor® 546 (sc-7546 AF546), Alexa Fluor® 594 (sc-7546 AF594) or Alexa Fluor® 647 (sc-7546 AF647), 200 µg/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor® 680 (sc-7546 AF680) or Alexa Fluor® 790 (sc-7546 AF790), 200 µg/ml, for Near-Infrared (NIR) WB, IF and FCM.

In addition, VP16 (14-5) is available conjugated to biotin (sc-7546 B), 200 µg/ml, for WB, IHC(P) and ELISA.

APPLICATIONS

VP16 (14-5) is recommended for detection of VP16 and VP16 fusion proteins by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)] and immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

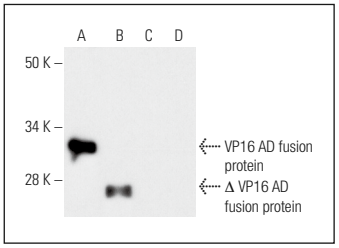
RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

DATA



VP16 (14-5): sc-7546. Western blot analysis of VP16. Full length transcriptional activation domain (AD) of VP16 (amino acids 412-490) fused to GAL4 (**A**), C-terminally deleted VP16 AD (amino acids 412-456) fused to GAL4 (**B**), N-terminally deleted VP16 AD (amino acids 452-490) fused to GAL4 (**C**) and GAL4 protein (**D**).

SELECT PRODUCT CITATIONS

1. Fotin-Mleczek, M., et al. 2000. Detection of protein-protein interactions using a green fluorescent protein-based mammalian two-hybrid system. Biotechniques 29: 22-26.
2. Geisen, C. and Moroy, T. 2002. The oncogenic activity of cyclin E is not confined to Cdk2 activation alone but relies on several other, distinct functions of the protein. J. Biol. Chem. 277: 39909-39918.
3. Tanaka, T. and Rabbitts T.H. 2003. Intrabodies based on intracellular capture frameworks that bind the Ras protein with high affinity and impair oncogenic transformation. EMBO J. 22: 1025-1035.
4. Narayanan, A., et al. 2005. Combinatorial transcription of herpes simplex virus and varicella zoster virus immediate early genes is strictly determined by the cellular coactivator HCF-1. J. Biol. Chem. 280: 1369-1375.
5. Bai, S., et al. 2008. Epidermal-growth-factor-dependent phosphorylation and ubiquitinylation of MAGE-11 regulates its interaction with the androgen receptor. Mol. Cell. Biol. 28: 1947-1963.
6. Clouaire, T., et al. 2010. Recruitment of MBD1 to target genes requires sequence-specific interaction of the MBD domain with methylated DNA. Nucleic Acids Res. 38: 4620-4634.
7. Levay, K. and Slepak, V.Z. 2014. Regulation of Cop9 signalosome activity by the EF-hand Ca^{2+}-binding protein tescalcin. J. Cell Sci. 127: 2448-2459.
8. Yamada, K., et al. 2016. The *Arabidopsis* CERK1-associated kinase PBL27 connects chitin perception to MAPK activation. EMBO J. 35: 2468-2483.

RESEARCH USE

For research use only, not for use in diagnostic procedures.