

P06002 DUALmembrane Control Extracts for Western Blotting

Contents: 2 tubes with 50 µl each of total extract
Size: 50 µl per tube, sufficient for 5 lanes

Contents and storage

Upon receipt, please store the individual components as indicated below.

DUALmembrane Control Extracts for Western Blotting

Control bait extract (DSY-5 transformed with pMBV1-Ost1)

- Store at -20°C

Control prey extract (DSY-5 transformed with pAl-Alg5)

- Store at -20°C

Introduction

The DUALmembrane control extract set contains total extracts made from the *Saccharomyces cerevisiae* strain DSY-5 transformed with (1) the plasmid pMBV1-Ost1, encoding the entire open reading frame of the *S. cerevisiae* gene Ost1 (YJL002C) fused to the Cub-LexA-VP16 reporter cassette and (2) the plasmid pAl-Alg5, encoding the entire open reading frame of the *S. cerevisiae* gene Alg5 (YPL227C) fused to the HA tag and the Nubl cassette.

Control bait extract

The fusion protein Ost1-Cub-LexA-VP16 is a typical DUALmembrane bait and can be used as a positive control when detecting DUALmembrane baits by Western Blotting. Visualization using an anti LexA antibody or an anti VP16 antibody yields a band around 100-120 kDa (there may be some heterogeneity in size due to variable glycosylation of Ost1).

Control prey extract

The fusion protein Alg5-Nubl is a DUALmembrane control prey and can be used as a positive control when detecting DUALmembrane preys by Western Blotting. Visualization using an anti HA antibody yields a band at 45 kDa.

Western Blotting procedure

- Prepare a SDS-PAGE gel
- Load 10 µl of each control extract along with your samples

- Run gel and transfer to a nitrocellulose or PVDF membrane by Western blotting
- To visualize the control proteins, use either an anti LexA or an anti VP16 antibody (for the bait control extract) or an anti HA antibody (for the control prey extract)

We recommend mouse monoclonal anti LexA antibody (sc-7544, Santa Cruz Biotechnology) and mouse monoclonal anti HA (MMS-101P, Covance Research Products).

Note

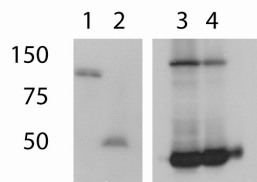
pMBV1-Ost1 is a low copy (CEN/ARS) plasmid with a low level CYC1 promoter. For this reason, the expression level of Ost1-Cub-LexA-VP16 is very low and you may need a highly sensitive reagent such as the SuperSignal West Femto substrate (Cat.-No. 34094, Pierce). We recommend the use of 0.5 ml substrate per Western blot (based on a standard Minigel, BioRad). Mix 0.25 ml of luminol/enhancer solution with 0.25 ml peroxide solution and carefully overlay onto the membrane, making sure that every part of the membrane is covered in solution. Incubate for 2-5 minutes and blot off excess liquid using a tissue. Wrap the membrane in saran wrap and expose to autoradiography film.

Expected Results

The control extracts should yield bands of the following sizes:

Ost1-Cub-LexA-VP16: 100-120 kDa

Alg5-Nub: 45 kD



Western blot of control extracts. Lanes: (1) 10 µl of control bait extract, (2) empty bait vector pBT3-N, (3-4) 10 µl control prey extract. Lanes 1-2: blotted with anti LexA antibody (sc-7544, Santa Cruz). Lanes 3-4: blotted with anti HA antibody (MMS-101 P, Covance Research Products).

Related products

DUALmembrane kit (P01001)

HTX β-galactosidase assay kit (P01002)

DS Yeast transformation kit (P01003)

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