**Product datasheet**

**Anti-GAL4 antibody - ChIP Grade ab1396**

**Overview**

Product name: Anti-GAL4 antibody - ChIP Grade  
Description: Rabbit polyclonal to GAL4 - ChIP Grade  
Host species: Rabbit  
Specificity: Customers feedbacks suggests that this antibody would not provide satisfactory results in Drosophila melanogaster.

**Tested applications**

Suitable for: WB, ChIP  
Species reactivity: Reacts with: Saccharomyces cerevisiae  
Does not react with: Drosophila melanogaster

**Immunogen**

Synthetic peptide corresponding to Saccharomyces cerevisiae GAL4 aa 100-200 conjugated to keyhole limpet haemocyanin.  
(Peptide available as ab23612)

**Positive control**

This antibody gave a positive signal with GAL4-VP16 recombinant protein.

**Properties**

Form: Liquid  
Storage instructions: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.  
Storage buffer: Preservative: 0.02% Sodium Azide  
Constituents: 1% BSA, PBS, pH 7.4  
Purity: Immunogen affinity purified  
Clonality: Polyclonal  
Isotype: IgG

**Applications**

Our Abpromise guarantee covers the use of ab1396 in the following tested applications.  
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
Relevance

Function: This protein is a positive regulator for the gene expression of the galactose-induced genes such as GAL1, GAL2, GAL7, GAL10, and MEL1 which encode for the enzymes used to convert galactose to glucose. It recognizes a 17 base pair sequence in (5'-CGGRNNRCYNNCNC-3') the upstream activating sequence (UAS-G) of these genes.

Subunit structure: Binds DNA as a homodimer. Interacts directly with the mediator subunits GAL11/MED15 and SRB4/MED17.

Domain: The 9aaTAD motif (residues 862 to 870) is a transactivation domain present in a large number of yeast and animal transcription factors.

Post-translational modification: Association between GAL11 and GAL4 may serve to expedite phosphorylation of GAL4.

Cellular localization

Nuclear

Application | Abreviews | Notes
--- | --- | ---
WB | Use a concentration of 1 µg/ml. Predicted molecular weight: 99 kDa. | 
ChIP | Use at an assay dependent dilution. | 

Images
Developed using the ECL technique. Performed under reducing conditions with exposure time of 5 mins. The samples run on a 4-20% gradient gel. All blocking and antibody incubation steps done in 5% milk in 20mM Tris-HCl plus 0.1% TWEEN-20.

Sample 1: Marker. Sample 2A: Whole cell yeast lysate control from untransformed SEY6210 pep4-3 strain (a kind gift from Prof. Tom Stevens from University of Oregon). Sample 2B: Whole cell yeast lysate from SEY6210 pep4-3 strain transformed with pJK22-pGBDU plasmid containing GAL4 DNA Binding domain fused to the VPS60 Gene (a kind gift from Prof. Tom Stevens from University of Oregon). Sample 2C: Whole cell yeast lysate from SEY6210 pep4-3 strain transformed with pJk23-pGAD plasmid containing GAL4 Active Domain fused to the VPS60 Gene (a kind gift from Prof. Tom Stevens from University of Oregon). Primary: Lane 1: none. Lane 2: Anti-GAL4BD (ab1396) antibody at 1ug/ml. Secondary: HRP conjugated Goat anti-Rabbit secondary antibody at 1/10000 dilution. Predicted band size: 99 kDa. Additional bands: 56 kDa (possible isoform, Fusion Protein)

A stably transfected 293T human cell line harbouring the GAL4 upstream activation sequence was transiently transfected with a V5 or T7- tagged GAL4 DNA Binding Domain construct. 48 hours post transfection Chromatin was prepared according to the Abcam X-ChIP protocol. The ChIP was performed with 25 ug chromatin, 5ug of antibody and 20 ul of Protein A/G beads. A non-specific antibody was used as the negative control. The immunoprecipitated DNA was quantified by real time PCR (SYBR Green approach).
S. cerevisiae cells were incubated in raffinose-containing media then transferred to galactose-containing media to activate transcription of galactose activated genes. IP was performed with 500μl of cell extract incubated overnight with 5μl of ab1396 at 4°C, followed by the addition of 25μl of protein A sepharose beads and incubation for 2 hours (room temp). The subsequently purified DNA was analysed with 3 pairs of primers (see diagram). The results show that Gal4 binds specifically to the UAS region of Gal1-Gal10 in both raffinose and galactose, despite only 500-600bp between the UAS and 5' primer.

Anti-GAL4 antibody - ChIP Grade (ab1396) at 1 µg/ml + GAL4-VP16 Recombinant Protein at 0.1 µg

**Secondary**
Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

**Predicted band size:** 99 kDa
**Observed band size:** 32 kDa

**Exposure time:** 2 minutes

ab1396 was tested against GAL4-VP16 Recombinant Protein predicted to run at 28 kDa.

---

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"
• Response to your inquiry within 24 hours

• We provide support in Chinese, English, French, German, Japanese and Spanish

• Extensive multi-media technical resources to help you

• We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit [https://www.abcam.com/abpromise](https://www.abcam.com/abpromise) or contact our technical team.

**Terms and conditions**

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors