

AM-Tag antibody (pAb)

Catalog Nos: 61677, 61678

RRID: AB_2793731 Isotype: IgG Application(s): ChIP, WB Reactivity: Human, Not Species Specific Quantities: 100 µg, 10 µg Purification: Protein A Chromatography Host: Rabbit Concentration: 1 µg/µl Molecular Weight: 15 kDa

Background: The **AM-Tag antibody** is specifically designed for use with Active Motif's unique AM-Tag sequence in combination with the Tag-ChIP-IT[®] Kit (Catalog No. 53022). Simply clone your protein of interest into Active Motif's pAM_1C Empty Vector (Catalog No. 53023), or add the AM-Tag sequence to the C-terminus of your protein expression vector. Following transfection, cell lysates are prepared and screened by Western blot for expression of the AM-Tag fusion protein using the AM-Tag antibody. Optimization may be required to identify the optimal conditions for transfection and protein expression.

Once transfection conditions have been optimized, the Tag-ChIP-IT[®] Kit can be used to isolate chromatin and perform ChIP. The AM-Tag has minimal cross reactivity with mammalian samples to ensure low background signal. The tag is also unstructured, which allows the tag to protrude from the protein of interest for maximum exposure during immunoprecipitation. This increases the enrichment efficiency of low abundance transcription factors for more reliable and consistent ChIP results.

Use of the AM-Tag for chromatin immunoprecipitation avoids the need for protein-specific ChIP-validated antibodies. It also enables the analysis of sequence variants, mutations and truncations on gene regulation.

Immunogen: This antibody was raised against Active Motif's unique AM-Tag sequence for use with Tag-ChIP-IT[®] (Cat No. 53022) analysis.

Buffer: Purified IgG in PBS with 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic.

Application Notes:

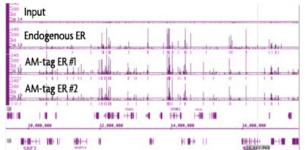
Validated Applications: ChIP: 10 µg per ChIP WB: 2 - 4 µg/ml dilution

Storage and Guarantee: Some products may be shipped at room temperature. This will not affect their stability or performance. Upon receipt, unconjugated antibodies may be stored at -20°C for up to 2 years. Fluorophore- & enzyme-conjugated antibodies should be stored at 4°C. Fluorophore-conjugated antibodies should be protected from light. Keep reagents on ice when not in storage; to avoid repeated freeze/thaw cycles, we recommend aliquoting items that will be stored frozen into single-use fractions prior to freezing. This product is guaranteed for 6 months from date of receipt.

This product is for research use only and is not for use in diagnostic procedures.

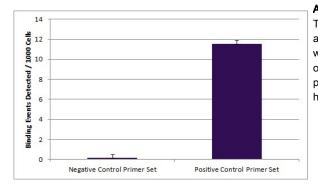
Application Key: ChIP = Chromatin Immunoprecipitation; FACS = Flow Cytometry; IF = Immunofluorescence; IHC = Immunohistochemistry; IP = Immunoprecipitation; WB = Western Blot





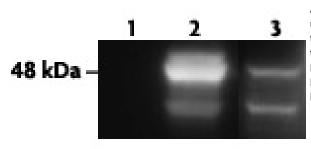
AM-Tag antibody (pAb)tested by ChIP-Seq.

Estrogen Receptor (ER) cDNA was cloned into pAM_1C Empty Vector (Catalog No. 53023) and sequence verified. Transient transfections were performed into Ishikawa cells. Cells were induced with estradiol and chromatin was harvested according to the instructions in the Tag-ChIP-IT® Kit (Catalog No. 53022). The AM-Tag antibody was used to immunoprecipitate the cross-linked AM-Tag-ER fusion protein. Following reversal of cross-links, enriched DNA was submitted for next-generation sequencing. Data was compared to published ChIP-Seq results using an anti-ER antibody in the same cell line and induction conditions. ChIP-Seq data shows the same ER peak profile with the AM-Tag immunoprecipitation as endogenous ER.



AM-Tag antibody (pAb)tested by ChIP.

Transcription factor JunD cDNA was cloned into pAM_1C Empty Vector (Catalog No. 53023) and sequence verified. Transient transfections were performed into HCT116 cells. Chromatin was harvested according to the instructions in the Tag-ChIP-IT® Kit (Catalog No. 53022). 10 µg of the AM-Tag antibody was used to immunoprecipitate the cross-linked AM-Tag-JunD fusion protein. qPCR data shows enrichment of JunD with the positive control primer set designed to hg19_dna range=chr16:90061257-90061903.



AM-Tag antibody (pAb)tested by Western blot.

Active Motif's pAM_1C_JunD Vector (Catalog No. 53044) was transfected into HCT116 cells using 10 µg DNA and 30 µl FuGENE transfection reagent or mock transfected. 48 hours post-transfection nuclear lysates were prepared. 20 µg lysate were loaded per well. JunD exists as two distinct isoforms (10 & 48 kDa).

- Lane 1: Mock transfection + 1:250 dilution AM-Tag antibody.
- Lane 2: Transfected cells + 1:250 dilution AM-Tag antibody.
- Lane 3: Mock transfection + 1:1000 dilution JunD antibody.