

# AbFlex<sup>®</sup> AM-Tag antibody (rAb)

Catalog Nos: 91111, 91112 RRID: AB\_2793779 Application(s): ChIP, ELISA, WB Reactivity: Human, Not Species Specific Quantities: 100 µg, 10 µg Purification: Protein A Chromatography Host: Mouse Isotype: IgG2a Concentration: 1 µg/µl Molecular Weight: 15 kDa

**Background:** AbFlex<sup>®</sup> antibodies are recombinant antibodies (rAbs) that have been generated using defined DNA sequences to produce highly specific, reproducible antibodies. Each AbFlex antibody contains a 6xHis Tag, a Biotinylation Tag for enzymatic biotin conjugation using the biotin ligase, BirA, and a sortase recognition motif (LPXTG) to attach a variety of labels directly to the antibody including fluorophores, enzymatic substrates (HRP, AP), peptides, drugs as well as solid supports.

AbFlex<sup>®</sup> AM-Tag antibody was expressed as full-length IgG with mouse immunoglobulin heavy and light chains (IgG2a isotype) in mammalian 293 cells.

The **AM-Tag antibody** is specifically designed for use with Active Motif's unique AM-Tag sequence in combination with the Tag-ChIP-IT<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> (Cat No. 53022) analysis.

**Buffer:** Purified IgG in 140 mM Hepes, pH 7.5, 70 mM NaCl, 32 mM NaOAc, 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic.

# **Application Notes:**

Applications Validated by Active Motif: ChIP: 10 µg per ChIP WB: 0.5 - 2 µg/ml dilution Bead-based ELISA: 7 - 560 ng/ml

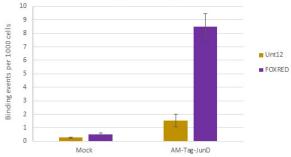
**Storage and Guarantee:** Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This product is guaranteed for 12 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



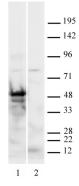
## AM-tag rAb ChIP qPCR



### AbFlex<sup>®</sup> AM-Tag antibody (rAb) tested by ChIP.

Active Motif's pAM\_1C\_JunD Vector (Catalog No. 53044) was transiently transfected or mock transfected into HCT116 cells. Chromatin was harvested according to the instructions in the Tag-ChIP-IT® Kit (Cat. 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 AM-Tag-JunD with the FOXRED qPCR primer set and little to no enrichment in the mock transfections or when using the negative control Unt12 qPCR primer set.



#### AbFlex<sup>®</sup> AM-Tag antibody (rAb) tested by Western Blot.

Active Motif's pAM\_1C\_JunD Vector (Catalog No. 53044) was transfected or mock transfected into HCT116 cells. 48 hours post-transfection nuclear lysates were prepared. 15 µg lysate was loaded per well. Western blot was performed using 1 µg/mL of AM-Tag antibody.