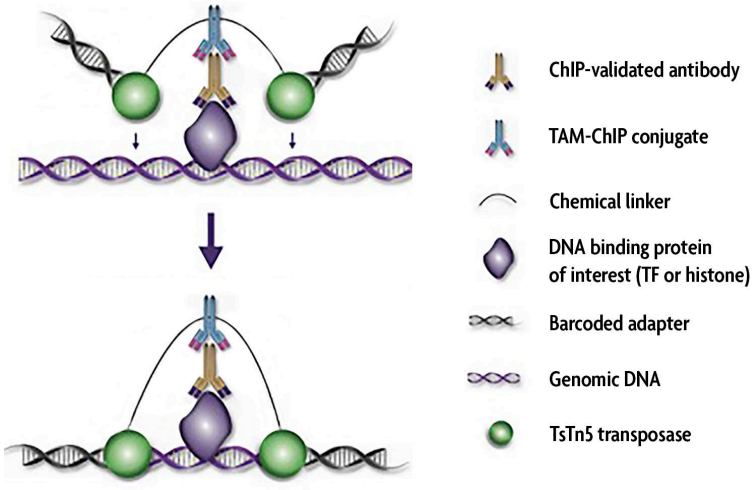
TAM-ChIP anti-rabbit conjugate

Catalog No: 53126
Lot No: 23517002
Format: 16 rxns

Backgound: Active Motif's TAM-ChIP combines antibody directed genomic targeting and NGS library preparation in one step. First, a ChIP-validated antibody is used to target a genomic region of interest, such as a histone or transcription factor binding site. Then, a TAM-ChIP anti-species antibody that is conjugated to barcoded sequencing adapters and TsTn5 transposase is added to each reaction. Activation of the transposase cuts the nearby DNA and pastes the antibody associated adapters into the DNA sequence. Following immunoprecipitation, enriched DNA is ready for library amplification and sequencing using Illumina® platforms.

Contents	Storage	Concentrations for Lot 23517002	Volumes to assemble TAM-ChIP antibody conjugate	Volumes for Oligo assembly
• Anti-rabbit conjugate	4°C	1.2 mg/ml	3.3 µl	N/A
• Oligonucleotide	-20°C	100 µM	N/A	2 µl
• pMENTS AR	-20°C	100 µM	0.76 µl	2 µl
• TsTn5 transposase	-20°C	142.4 µM	0.53 µl	1.4 µl
• TAM-ChIP Index 1-16	-20°C	0.5 µM		
• Amplification primer mix	-20°C	10 µM ea		
• 5X Tagmentation Buffer	-20°C	5X		

Related Products (not included)
• TAM-ChIP Assay Reagents (Catalog No. 53128)



TAM-ChIP illustration.
Intact cells are fixed with formaldehyde to preserve protein/DNA interactions. DNA is then sheared using sonication and incubated with a ChIP-validated antibody directed against the protein of interest. A TAM-ChIP antibody conjugate containing Illumina-compatible sequencing adapters and transposase is added. Activation of the transposase cuts the DNA surrounding the genomic region of interest and pastes the barcoded adapters into the DNA sequences. The protein/DNA complexes are immunoprecipitated, DNA cross-links are reversed and proteins are degraded. ChIP enriched DNA is ready for library amplification and sequencing.

Quality Control: The TAM-ChIP anti-rabbit conjugate is tested in combination with Active Motif's TAM-ChIP Assay Reagents (Catalog No. 53128) using 10 µg of MCF-7 chromatin in combination with 4 µg CTCF antibody and 4 µg of assembled TAM-ChIP anti-rabbit conjugate according to the instructions provided below. Enriched DNA is PCR amplified and size selected. ChIP-Seq is performed using the Illumina NextSeq 500 and sequencing results are mapped to the reference genome.

Protocol Guidelines: The following guidelines are provided for the use of the TAM-ChIP anti-mouse conjugate. Use only the values supplied on the appropriate lot-specific data sheet. If using in conjunction with TAM-ChIP assay reagent (Cat. No. 53128), please follow that manual and the lot-specific information on page 1.

Sample Preparation:

1. Prepare chromatin from cultured cell lines or tissue samples using your preferred protocol or Active Motif's TAM-ChIP Assay Reagents (Catalog No. 53128). We suggest formaldehyde fixation (1% final concentration) for 15 minutes at room temperature followed by addition of a glycine stop solution for 5 minutes at room temperature.

Note: Due to the sensitivity of the TsTn5 transposase to SDS, it is important that buffers used during cell lysis and chromatin sonication do not contain SDS concentrations that exceed 0.1% SDS. The maximum amount of SDS that can be used in the immunoprecipitation reaction should not exceed 0.02% final concentration.

2. Sonicate chromatin to achieve a sheared size of 200-1000 bp. To prevent overheating and denaturation of chromatin, samples should be kept on ice as much as possible during shearing, and shearing should be performed discontinuously (*e.g.* sonicate for 20 seconds, then place on ice for 30 seconds before sonicating again for 20 seconds, etc.)
3. Remove a 25 µl sample of sonicated chromatin for chromatin quantification. Reverse the protein cross-links and Proteinase K treat the chromatin sample. Read the absorbance on a NanoDrop or other spectrophotometer at 260nm to determine the DNA concentration. Use a Bioanalyzer or agarose gel to confirm the size of the sheared chromatin.

Recommendations for use with ChIP protocols other than TAM-ChIP:

1. We suggest performing the immunoprecipitation reaction using 5 - 10 µg chromatin and 4 µg ChIP-validated antibody per reaction. We also recommend setting up a ChIP reaction without primary antibody to serve as a tagmentation control. This control will show the effects of the transposase in the absence of genomic targeting and provide a background reference during sequencing. Incubate ChIP reactions on an end-to-end rotator at 4°C overnight.
2. The next day, prepare the TAM-ChIP antibody conjugate and the Oligo assembly using the lot-specific information provided on page 1 of the lot-specific data sheet. We recommend using an equivalent mass for the TAM-ChIP conjugate as the ChIP-Seq antibody (4 µg). Use separate tubes to prepare the antibody conjugate and the oligo assembly. The volumes provided are for 1 reaction, please scale up accordingly.

Antibody Conjugate

Reagent	**1 reaction**
TAM-ChIP Antibody conjugate	See page 1
pMENTS AM (100 µM)	See page 1
Incubate for 30 minutes at RT	
TsTn5 transposase	See page 1
Incubate for 1 hour at RT	

Oligo Assembly

Reagent	**1 reaction**
Oligonucleotide (100 µM)	See page 1
pMENTS AM (100 µM)	See page 1
Incubate for 30 minutes at RT	
TsTn5 transposase	See page 1
Incubate for 1 hour at RT (Keep at RT until ready to use)	

3. Add the 4 µg of the assembled TAM-ChIP anti-mouse conjugate to each ChIP reaction from Step 1. Incubate on an end-to-end rotator at 4°C for 1 hour.
4. Adjust the amount of DTT in your reaction to a final concentration of 0.35 mM and incubate an end-to-end rotator at 37°C for 1 hour.
5. Capture your ChIP reactions using Protein G beads. Wash to remove unbound DNA. The inclusion of 0.1% SDS in the wash buffer will improve your wash efficiency. Resuspend your ChIP reactions in 400 µl buffer without SDS.
6. Activate the TsTn5 transposase with the addition of 5X Tagmentation Buffer. Add 100 µl Tagmentation buffer to 400 µl ChIP reaction for a final volume of 500 µl.
7. Adjust the amount of DTT in your reaction to a final concentration of 0.35 mM (1.75 µl of 10 mM DTT stock).
8. Incubate on an end-to-end rotator at 37°C for 1 hour.
9. Wash beads and elute the chromatin.
10. Clean up the eluted DNA using phenol/chloroform and phase lock tubes followed by either ethanol precipitation or column purification. Elute in 25 µl 10 mM Tris-Cl, pH 8.5.

Library Amplification:

1. PCR amplify the ChIP enriched DNA. Below is an example PCR reaction. We strongly recommend using Q5 High-Fidelity polymerase (New England Biolabs, M0491) to ensure good amplification efficiency. Other polymerase mixtures may require optimization for efficient amplification. Follow the recommendations of your manufacturer's PCR reagents.

Reagent	**1 reaction**
ChIP DNA	20 µl
TAM-Index primer* (0.5 µM)	5 µl
Amplification primer mix (10 µM each)	5 µl
5X Q5 Reaction Buffer	10 µl
Q5 High-Fidelity Polymerase	0.5 µl
dNTPs (10 mM)	1 µl
Sterile H_2O	8.5 µl
Total volume	**50 µl**

*If combining multiple TAM-ChIP samples together for sequencing, make sure to use unique TAM-ChIP Index primers for each sample.

Thermal Cycler Program:
98°C for 30 seconds
63°C for 3 minutes

98°C for 10 seconds
63°C for 30 seconds x 14 cycles
72°C for 30 seconds

4°C Hold

Proceed immediately to Library Size Selection

2. Size select the ChIP libraries using AMPure beads and 70% ethanol to 200-500 bases.
3. Assess chromatin size using TapeStation or equivalent method.
4. Sequence using an Illumina platform.

For sequencing, we recommend 30 million reads. If combining multiple TAM-ChIP samples together for sequencing, make sure to use unique TAM-ChIP Index primers for each sample. The TAM-ChIP Index (i7) contains an 8 bp random molecular identified (MID) followed by a 3 bp sample barcode for a total of 11 bp. The sequences below represent the true sequence and the NextSeq 500 sequence of index (i7). (If using different instruments determine if the true sequence or reverse complement should be entered).

	True Sequence	NextSeq 500 Sequence
TAM-ChIP Index 1	NNNNNNNNTCG	NNNNNNNNCGA
TAM-ChIP Index 2	NNNNNNNNGAC	NNNNNNNNGTC
TAM-ChIP Index 3	NNNNNNNNACG	NNNNNNNNCGT
TAM-ChIP Index 4	NNNNNNNNGCT	NNNNNNNNAGC
TAM-ChIP Index 5	NNNNNNNNTGA	NNNNNNNNTCA
TAM-ChIP Index 6	NNNNNNNNCGT	NNNNNNNNACG
TAM-ChIP Index 7	NNNNNNNNCTA	NNNNNNNNTAG
TAM-ChIP Index 8	NNNNNNNNGTA	NNNNNNNNTAC
TAM-ChIP Index 9	NNNNNNNNAGT	NNNNNNNNACT
TAM-ChIP Index 10	NNNNNNNNATC	NNNNNNNNGAT
TAM-ChIP Index 11	NNNNNNNNTAG	NNNNNNNNCTA
TAM-ChIP Index 12	NNNNNNNNCAG	NNNNNNNNCTG
TAM-ChIP Index 13	NNNNNNNNTAC	NNNNNNNNGTA
TAM-ChIP Index 14	NNNNNNNNGTC	NNNNNNNNGAC
TAM-ChIP Index 15	NNNNNNNNCAC	NNNNNNNNGTG
TAM-ChIP Index 16	NNNNNNNNACA	NNNNNNNNTGT

The antibody index (i5) is an 8 bp sequence of which 3 bp are used for analysis. The true sequence for the anti-mouse conjugate i5 index is ATA and the NextSeq 500 sequence is TAT.

We recommend running the machine in standalone mode to allow use of different length sequencing of the i5 and i7 indexes (11 on i7 and 8 on i5). If using BaseSpace, you will need the same number of reads on each end (11 and 11). The type of sequencing to run may depend on your instrument, cartridge capacity and application. We routinely perform singe-end sequencing, 73 cycles.

For additional information regarding data analysis and de-duplication of the MIDs, please visit Active Motif's website www.activemotif.com/tamchip and download the Guidelines for TAM-ChIP Molecular Identifier (MID) Analysis from the "Documents" tab. Or contact Technical Support at tech_service@activemotif.com

Guarantee: This product is for research use only and is not for use in diagnostic procedures. This product is guaranteed for 6 months from date of arrival.