

# High Throughput CHIP-IT® Kit Manual

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(version A1)

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## Overview

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The High Throughput ChIP-IT® Kits are designed for use with chromatin that has been prepared using the PIXUL Chromatin Shearing Kit (Cat. No. 53132) or the High Sensitivity Chromatin Preparation Kit (Cat. No. 53134).

We recommend using chromatin derived from 10,000 to 750,000 cell equivalents (approximately 70 ng to 3.5 µg, based on the mass of DNA per cell in a diploid human genome) per reaction.

For histone modification IP the chromatin should represent a minimum of 10,000 cell equivalents (70 ng of chromatin), and for transcription factor IP the chromatin should represent a minimum of 50,000 cell equivalents (333 ng).

### High Throughput ChIP-IT® Kit Advantages

- Enables rapid and efficient processing of a larger number of ChIP samples
- Compatible with chromatin sheared with PIXUL or other traditional methods

product	format	catalog no.
High Throughput ChIP-IT® Kit	24 reactions	53146

## Kit Components and Storage

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The kit contains sufficient reagents for 24 ChIP reactions. The reagents in this kit have multiple storage temperatures. Please store components according to the storage conditions below. All reagents are guaranteed stable for 6 months from date of receipt when stored properly.

### High-Throughput ChIP Kit Reagents

Reagents	Quantity	Storage
Protein G Magnetic Beads	1.1 mL	4°C
TE, pH 8.0	30 mL	RT
Protease Inhibitor Cocktail	60 µL	-20°C
Blocking Agent AM1	96 µL	-20°C
BSA (10 mg/mL)	96 µL	-20°C
5X ChIP Buffer	0.6 mL	RT
ChIP Buffer	10 mL	RT
Blocker	312 µL	-20°C
Wash Buffer AM1	9 mL	RT
LiCl Buffer	9 mL	RT
Elution Buffer AM4	1.2 mL	RT
Proteinase K (10 µg/µL)	48 µL	-20°C
RNase A (10 µg/µL)	24 µL	-20°C
Low EDTA TE	1 mL	RT
SPRI Beads	2 X 1 mL	4°C
Bar Magnet & Glue Dots	1 bar & 2 strips of Glue Dots	RT

### **Additional Materials Required**

- 100% Ethanol
- Antibodies for ChIP reactions
- DNase-free water
- Microcentrifuge tubes
- 250  $\mu$ L PCR 8-tube strips
- Microcentrifuge
- Benchtop centrifuge
- Tube rotator
- Vortexer
- Thermal cycler
- Multichannel pipette (20 - 200  $\mu$ L)
- Pipette tips

# High Throughput ChIP-IT® Kit Protocol

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This Kit is optimized for chromatin that has been prepared using the PIXUL Chromatin Shearing Kit (Cat. No. 53132). We recommend sonicating at least 500,000 cells per sample when using the High Throughput ChIP-IT® Kit.

**NOTE: We recommend preparing the pre-clearing beads and the IP beads in parallel. The pre-clearing beads will be used on day 1 and the beads used in the IP will incubate overnight and be used on day 2. These can be prepared up to one week ahead of performing the chromatin IP itself.**

## Section A. Prepare Beads for Pre-Clearing (Volumes Per Reaction)

1. Transfer 10  $\mu$ L per reaction of well-mixed Protein G Magnetic Beads into a 200  $\mu$ L PCR tube.
2. Collect beads using a magnet, aspirate supernatant, and add 10  $\mu$ L of TE pH 8.0 to wash the beads.
3. Briefly vortex or pipette to mix, and centrifuge briefly to remove liquid from the tube cap.
4. Collect beads using a magnet, aspirate supernatant and add:  
8  $\mu$ L TE, pH 8.0  
1  $\mu$ L Blocking Agent AMI  
1  $\mu$ L BSA
5. Incubate for 2 hours at 4°C on a rotator, ensuring the solution is mixing within the tube. Incubation can be extended to 4 hours if desired.
6. Centrifuge briefly to remove liquid from the tube cap, apply the magnet to collect beads, aspirate the supernatant, and resuspend beads in 10  $\mu$ L ChIP Buffer.
7. Briefly pipette to mix, and centrifuge briefly to remove liquid from the cap.
8. Collect beads using a magnet, aspirate supernatant, and add 10  $\mu$ L of ChIP Buffer to resuspend beads. The beads are now ready for pre-clearing chromatin.

## Section B. Prepare Beads for Immunoprecipitation (Volumes Per Reaction)

1. Mix Protein G Magnetic Beads well and transfer 30  $\mu$ L into a tube.
2. Collect beads using a magnet, aspirate supernatant, and add 10  $\mu$ L TE, pH 8.0.
3. Briefly vortex or pipette to mix, and centrifuge briefly to remove liquid from the cap.
4. Collect beads using a magnet, aspirate supernatant, and add to each:  
21  $\mu$ L TE, pH 8.0  
3  $\mu$ L Blocking Agent AMI  
3  $\mu$ L BSA  
3  $\mu$ L Blocker
5. Incubate overnight at 4°C on a rotator, ensuring the solution is mixing in the tube.

## Section C. Chromatin Immunoprecipitation Assay

Chromatin used in this High Throughput ChIP-IT Kit protocol should represent a minimum of 10,000 cell equivalents (70 ng chromatin) for histone modification IP, and 50,000 cell equivalents (333 ng) for transcription factor IP.

Active Motif's ChIP Buffer has been optimized to yield specific and sensitive immunoprecipitation. We recommend that chromatin, regardless of source, be immunoprecipitated in a volume of 100-150  $\mu$ L 1X ChIP Buffer.

1. Thaw Protease Inhibitor Cocktail on ice. If the chromatin sample was stored at  $-80^{\circ}\text{C}$ , thaw on ice. Once thawed, if the chromatin needs to be diluted, adjust the volume of chromatin with 5X ChIP Buffer and DNase-free water to a final concentration of 1X ChIP Buffer. For example, 50  $\mu$ L chromatin (140 ng/ $\mu$ L) + 30  $\mu$ L water + 20  $\mu$ L 5X ChIP Buffer = 100  $\mu$ L chromatin (70 ng/ $\mu$ L).

**NOTE:** If PIXUL-sonicated chromatin is used, add 25  $\mu$ L of 5X ChIP Buffer to 100  $\mu$ L of chromatin to yield a 1X ChIP Buffer solution for IP.

2. Add 2.5  $\mu$ L of Protease Inhibitor Cocktail to each reaction.
3. Thoroughly mix Pre-Clearing Beads that were prepared in Section A above. Add 10  $\mu$ L of Pre-Clearing Beads to each reaction.
4. Cap tubes or plate well and incubate for 3 hours at  $4^{\circ}\text{C}$  on a rotator. 30 minutes before this incubation is complete, thaw out the Blocker and prepare the antibody & Blocker for IP in the next step while the pre-clearing step completes.

**NOTE:** If the prepared chromatin is representative of less than 100,000 cell equivalents or less than 1  $\mu$ g, per 100  $\mu$ L of chromatin, do not add blocker to antibody.

5. Add 2 to 4  $\mu$ g of desired antibody and 10  $\mu$ L of Blocker to new tubes, pipette to mix, and incubate at room temperature for 10 to 20 minutes.
6. Briefly centrifuge pre-cleared chromatin tubes or plate that has completed the incubation in Step 4 above, and place on magnet for 5 minutes to pellet beads.
7. Remove caps and transfer the cleared chromatin to the antibody-containing tubes from Step 5.
8. Cap tubes or plate tightly, and incubate overnight at  $4^{\circ}\text{C}$  on a rotator.
9. Briefly centrifuge tubes or plate, remove caps, and add 30  $\mu$ L of Beads for Immunoprecipitation that were prepared in Section B above. Incubate for 3 hours at  $4^{\circ}\text{C}$  on a rotator.
10. Briefly centrifuge tubes or plate to remove liquid from caps, and place tubes or plate on magnet to pellet beads.
11. Remove caps, and aspirate liquid. Remove tubes or plate from magnet, and add 180  $\mu$ L of ChIP Buffer to each well, wait 30 seconds and place tubes or plate back on the magnet.

While on the magnet, rotate the tubes or plate 180 degrees every 30 seconds for a total of 4 rotations. The rationale for this type of wash step throughout this protocol is to move the beads through the solution to facilitate dissociation of non-specifically bound material.

12. Repeat step 11: Remove caps, and aspirate liquid. Remove tubes or plate from magnet, and add 180  $\mu$ L of CHIP Buffer to each well, wait 30 seconds and place tubes or plate back on the magnet. While on the magnet, rotate the tubes or plate 180 degrees every 30 seconds for a total of 4 rotations.
13. Remove caps, and aspirate liquid. Remove tubes or plate from magnet, and add 180  $\mu$ L of Wash Buffer AM1 to each well, wait 30 seconds and place tubes or plate back on the magnet. While on the magnet, rotate the tubes or plate 180 degrees every 30 seconds for a total of 4 rotations.
14. Repeat Step 13: Remove caps, and aspirate liquid. Remove tubes or plate from magnet, and add 180  $\mu$ L of Wash Buffer AM1 to each well, wait 30 seconds and place tubes or plate back on the magnet. While on the magnet, rotate the tubes or plate 180 degrees every 30 seconds for a total of 4 rotations.
15. Remove caps, and aspirate liquid. Remove tubes or plate from magnet, and add 180  $\mu$ L of LiCl Buffer to each well, wait 30 seconds and place tubes or plate back on the magnet. While on the magnet, rotate the tubes or plate 180 degrees every 30 seconds for a total of 4 rotations.
16. Repeat Step 15: Remove caps, and aspirate liquid. Remove tubes or plate from magnet, and add 180  $\mu$ L of LiCl Buffer to each well, wait 30 seconds and place tubes or plate back on the magnet. While on the magnet, rotate the tubes or plate 180 degrees every 30 seconds for a total of 4 rotations.
17. Remove caps, and aspirate liquid. Remove tubes or plate from magnet, and add 180  $\mu$ L of TE, pH 8.0 to each well, wait 30 seconds and place tubes or plate back on the magnet. While on the magnet, rotate the tubes or plate 180 degrees every 30 seconds for a total of 4 rotations.
18. Repeat Step 17: Remove caps, and aspirate liquid. Remove tubes or plate from magnet, and add 180  $\mu$ L of TE, pH 8.0 to each well, wait 30 seconds and place tubes or plate back on the magnet. While on the magnet, rotate the tubes or plate 180 degrees every 30 seconds for a total of 4 rotations.
19. Completely aspirate TE, and add 50  $\mu$ L Elution Buffer AM4.
20. Cap tubes or plate tightly, vortex to mix, briefly centrifuge, and incubate for 15 minutes at 37°C.
21. Briefly centrifuge tubes or plate, place on magnet and let stand on magnet for 5 minutes. Uncap tubes or plate, and transfer supernatant containing immunoprecipitated chromatin to a new PCR tube or plate.



## Section D. Reverse Crosslinking

1. Add 10  $\mu$ L TE pH 8.0 and 1  $\mu$ L of RNase A to each sample. Vortex the samples to mix, then centrifuge briefly. Incubate for 20 minutes at 37°C.
2. Centrifuge briefly and add 2  $\mu$ L of Proteinase K to each sample. Cap tubes or plate tightly, vortex briefly to mix, and briefly centrifuge. Incubate for 2 hours at 50°C, followed by an overnight incubation at 65°C in a thermal cycler.

## Section E. DNA Purification

**NOTE:** Reactions can be stored at 4°C or -20°C for up to a week if not proceeding directly to the DNA purification steps below. If samples are stored, it is **CRITICAL** to heat the samples to 50°C prior to adding SPRI beads in order to redissolve any precipitate.

1. Mix SPRI beads well, and add 80  $\mu$ L SPRI beads to each sample. Pipette to mix, and incubate at room temperature for 5 minutes.
2. Place tubes or plate on magnet for 5 minutes.
3. Carefully aspirate supernatant, and add 180  $\mu$ L fresh 80% ethanol to wash beads.
4. Leave the plate on the magnet, carefully aspirate supernatant, and add another 180  $\mu$ L fresh 80% ethanol.
5. Completely aspirate supernatant and let beads air dry until they are no longer shiny (30 seconds to 2 minutes). Do not over-dry the beads to the point that they become cracked. Over-drying makes reconstitution of beads and elution of DNA more difficult.
6. Add 40  $\mu$ L Low EDTA TE to each tube or well of plate, and pipette to mix.
7. Incubate at room temperature for 3 minutes, place tubes or plate on magnet to separate beads, and transfer supernatant containing ChIP-DNA to new tubes or new plate for analysis.

## Analysis of Immunoprecipitated Chromatin

If ChIP-Seq is going to be performed, we recommend preparing libraries with the Next Gen DNA Library Kit (Cat. No. 53216). If ChIP-qPCR is going to be performed, we recommend the ChIP-IT® qPCR Analysis Kit (Cat. No. 53029).

# Troubleshooting Guide

Problem/question	Possible cause	Recommendation
Low signal or high background	Antibody performance	<p>A positive control reaction targeting a prevalent chromatin constituent using a ChIP-grade antibody is recommended, such as H3K4me3.</p> <p>If the epitope of interest is enzymatically modifiable, it is highly recommended that an appropriate inhibitor be included, beginning from sonication. For example, include an HDAC inhibitor in an IP for H3K27ac.</p> <p>If the target is of low or uncertain abundance, increasing the mass of chromatin per reaction can improve results.</p>
Poor ChIP-Seq results	Low yield or persistent high molecular weight fragments	Avoid over-fixation. Over-fixation can lead to the formation of heavily crosslinked, condensed chromatin that is resistant to shearing and difficult to solubilize, reducing yield.
	Fragments not in ideal range	<p>We find that fragments in the 200-1200 bp size range perform best in ChIP, and recommend that the size distribution of input chromatin be inspected by electrophoretic methods in order to validate shearing.</p> <p>Biological samples vary greatly in ways that impact their response to ultrasound. When sonicating a new sample type, optimization of sonication is recommended before attempting a ChIP-Seq experiment.</p>

## Technical Services

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If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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