High Throughput ChIP-IT® Kit

Catalog No. 53145 (96 rxns)

Catalog No. 53146 (24 rxns)

(version A2)

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Overview

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 71 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 (67 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

- Process up to 96 samples at a time in PCR strip tubes or a 96-well plate
- Compatible with chromatin sheared with PIXUL or other traditional methods

product	format	catalog no.
High Throughput ChIP-IT® Kit	96 reactions	53145
High Throughput ChIP-IT® Kit	24 reactions	53146

Kit Components and Storage

The kit contains sufficient reagents for 24 or 96 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 24 rxns/96 rxns	Storage
Protein G Magnetic Beads	1.5 mL / 4 X 1.1 mL	4°C
TE, pH 8.0	35 mL / 2 X 35 mL	RT
Protease Inhibitor Cocktail	105 µL / 500 µL	-20°C
Blocking Agent AM1	120 µL / 450 µL	-20°C
BSA (10 mg/mL)	120 µL / 450 µL	-20°C
5X ChIP Buffer	1.5 mL / 2 X 1.5 mL	RT
ChIP Buffer	35 mL / 50 mL	RT
Blocker	500 µL / 3 X 500 µL	-20°C
Wash Buffer AM1	30 mL / 100 mL	RT
LiCl Buffer	40 mL / 40 mL	RT
Elution Buffer AM4	1.6 mL / 12 mL	RT
Proteinase K (10 µg/µL)	86 µL / 2 X 200 µL	-20°C
RNase A (10 µg/µL)	45 µL / 3 X45 µL	-20°C
Low EDTA TE	1.2 mL / 7 mL	RT
SPRI Beads	2 X 1.1 mL / 2 X 4.5 mL	4°C
Bar Magnet & Glue Dots	1 bar & 2 strips of Glue Dots (24 rxns only)	RT

Additional Materials Required

- 100% Ethanol
- Antibodies for ChIP reactions
- DNase-free water
- Microcentrifuge tubes
- 250 µL PCR 8-tube strips
  or 96-well PCR plate with a seal that will prevent cross-contamination between wells
- Microcentrifuge
- Benchtop centrifuge
- Tube rotator
- Vortexer
- Thermal cycler
- Multichannel pipette (20 - 200 µL)
- Magnetic plate (if using 96-well PCR plate)
- Pipette tips

High Throughput ChIP-IT® Kit Protocol

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.

The following protocol provides recommendations for a full 96-well plate of ChIP reactions with 5% extra volume to account for loss during pipetting and aliquoting. Volumes for individual reactions are also provided and can be used for smaller batches.

If a 96-well plate is desired to be used, it is important to use a plate that can be securely sealed with caps so that the plate does not leak during the overnight rotation at 4°C.

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 µL per reaction of well-mixed Protein G Magnetic Beads into a 200 µL PCR tube.
2. Collect beads using a magnet, aspirate supernatant, and add 10 µL 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 µL TE, pH 8.0
   1 µL Blocking Agent AM1
   1 µL 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 µL 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 µL of ChIP Buffer to resuspend beads. The beads are now ready for pre-clearing chromatin.

Section A. Prepare Beads for Pre-Clearing (Volumes for Full 96-well Plate)

1. Transfer 1008 µL of well-mixed Protein G Magnetic Beads into a 2 mL tube.
2. Collect beads using a magnet, aspirate supernatant, and add 1 mL 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:

   806 µL TE, pH8.0
   101 µL Blocking Agent AM1
   101 µL 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, apply the magnet to collect beads, aspirate the supernatant, and resuspend beads in 1 mL 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 1008 µL 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 µL into a tube.
2. Collect beads using a magnet, aspirate supernatant, and add 10 µL 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 µL TE, pH 8.0
   3 µL Blocking Agent AM1
   3 µL BSA
   3 µL Blocker
5. Incubate overnight at 4°C on a rotator, ensuring the solution is mixing in the tube.

Section B. Prepare Beads for Immunoprecipitation (Volumes Per Full 96-well Plate)

1. Mix Protein G Magnetic Beads well and transfer into two 2 mL tubes 1512 µL per tube (3024 µL total).
2. Collect beads using a magnet, aspirate supernatant, and add 1 mL TE, pH 8.0 to each 2 mL tube.
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 2 mL tube:

   1060 µL TE, pH 8.0
   151 µL Blocking Agent AM1
   151 µL BSA
   151 µL 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 (67 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 µL 1X ChIP Buffer.

1. Thaw Protease Inhibitor Cocktail on ice. If the chromatin sample was stored at -80°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 µL chromatin (140 ng/µL) + 30 µL water + 20 µL 5X ChIP Buffer = 100 µL chromatin (70 ng/µL).

   NOTE: If PIXUL-sonicated chromatin is used, add 25 µL of 5X ChIP Buffer to 100 µL of chromatin to yield a 1X ChIO Buffe solution for IP.

2. Add 2.5 µL of Protease Inhibitor Cocktail to each reaction.
3. Thoroughly mix Pre-Clearing Beads that were prepared in Section A above. Add 10 µL of Pre-Clearing Beads to each reaction.
4. Cap tubes or plate well and incubate for 3 hours at 4°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 µg, per 100 µL of chromatin, do not add blocker to antibody.

5. Add 2 to 4 µg of desired antibody and 10 µL 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°C on a rotator.
9. Briefly centrifuge tubes or plate, remove caps, and add 30 µL of Beads for Immunoprecipitation that were prepared in Section B above. Incubate for 3 hours at 4°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 µL 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 µL 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 µL 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 µL 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 µL 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 µL 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 µL 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 µL 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 µL 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 µL TE pH 8.0 and 1 µL of RNase A to each sample, or for a full plate prepare 1008 µL TE pH 8.0 plus 100.8 µL RNase A and dispense 11 µL of this mix to each sample.. Vortex the samples to mix, then centrifuge briefly. Incubate for 20 minutes at 37°C.
2. Centrifuge briefly and add 2 µL 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 µL 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 µL fresh 80% ethanol to wash beads.
4. Leave the plate on the magnet, carefully aspirate supernatant, and add another 180 µL 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 µL 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	A positive control reaction targeting a prevalent chromatin constituent using a ChIP-grade antibody is recommended, such as H3K4me3. 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. If the target is of low or uncertain abundance, increasing the mass of chromatin per reaction can improve results.
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	We find that fragments in the 100-600 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. Biological samples vary greatly in ways that impact their response to ultrasound. When sonicating a new sample type, optimiaztion of sonication is recommended before attempting a ChIP-Seq experiment.

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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