ATAC-Seq Kit Manual

Catalog No. 53150

(version B7)

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Overview

The Assay for Transposase-Accessible Chromatin via Sequencing (ATAC-Seq) method was first introduced in 2013[1]. ATAC-Seq is a rapid assay of the epigenetic state by enabling identification of open chromatin regions. In the assay, intact nuclei are treated with a hyperactive Tn5 transposase mutant which is able to simultaneously tag the target DNA with sequencing adapters and fragment the DNA in a process termed "tagmentation"[2].

Because of the assay's speed, simplicity, sensitivity, and applicability to a wide range of sample types, ATAC-Seq has become a commonly used epigentic assay, and can serve as a gateway to further, more detailed, epigenetic analyses. The ATAC-Seq Kit provides the reagents necessary to produce 16 unique sequencing-ready Illumina®-compatible ATAC-Seq libraries from 20 - 30 mg tissue or 50,000 - 100,000 cells per reaction.

ATAC-Seq Advantages

- Assess the epigenetic profile of open chromatin regions
- Yields next-gen sequencing-ready processed samples in hours
- Simple and rapid three-step protocol

product	format	catalog no.
ATAC-Seq Kit	16 reactions	53150

Note: The ATAC-seq Kit is for research use only. Not for use in diagnostic procedures.

Kit Components and Storage

The kit contains sufficient reagents to produce 16 unique next-gen sequencing-ready ATAC-Seq libraries. The reagents in this kit have multiple storage temperatures. The ATAC-Seq Kit is shipped at two temperatures, with one box on dry ice for components to be stored at -20°C, and a second box at room temperature for components to be stored at 4°C. Please store components according to the storage conditions below. All reagents are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents for ATAC-Seq

Reagents	Quantity	Storage
ATAC Lysis Buffer	17 ml	RT
Assembled Transposomes	170 µl	-20°C
2X Tagmentation Buffer	425 µl	-20°C
1X PBS	2 x 1 ml	-20°C or RT
10X PBS	500 µl	RT
10% Tween 20	10 µl	RT
1.0% Digitonin	10 µl	RT
DNA Purification Columns	16 columns	RT
DNA Purification Binding Buffer	4.5 ml	RT
DNA Purification Wash Buffer	10 ml	RT
DNA Purification Elution Buffer	5 ml	RT
3 M Sodium Acetate	450 µl	RT
10 mM dNTPs	40 µl	-20°C
5X Q5 Buffer	2 x 130 µl	-20°C
Q5 High-Fidelity DNA Polymerase (2U/µl)	10 µl	-20°C
i7 Indexed Primer 1	10 µl	-20°C
i7 Indexed Primer 2	10 µl	-20°C
i7 Indexed Primer 3	10 µl	-20°C
i7 Indexed Primer 4	10 µl	-20°C
i5 Indexed Primer 1	10 µl	-20°C
i5 Indexed Primer 2	10 µl	-20°C
i5 Indexed Primer 3	10 µl	-20°C
i5 Indexed Primer 4	10 µl	-20°C
SPRI Beads	1 ml	4°C

Additional Materials Required

- 100% Ethanol
- Deionized water
- Trypan Blue
- Pipettes and corresponding pipette tips
- Microcentrifuge
- Swing-bucket centrifuge
- 1.5 ml microcentrifuge tubes
- 300 µl PCR tubes
- Bar magnet
- 40 µm filter (for tissue samples)
- Razor blade (for tissue samples)
- 5 cm petri dish (for tissue samples)
- 15 ml conical tubes (for tissue samples)
- Ice-cold PBS (for tissue samples)
- Dounce homogenizer (for tissue samples)

Note: The protocols below are designed for 20 to 30 mg tissue or 50,000 to 100,000 cells.

Tissue Sample Preparation

This protocol is designed for 20 to 30 mg of tissue per reaction. Fresh tissue can be used or tissue that has been flash frozen at -80°C.

Take SPRI beads out of 4°C and warm to room temperature by simply placing the vial of SPRI beads on the benchtop.

1. For each sample, label a 5 cm petri dish and place on wet ice, along with a labeled 15 ml conical tube containing 5 ml ice-cold PBS.
2. Transfer each sample to its corresponding dish, mince with a razor blade, and transfer to the corresponding 15 ml conical tube containing ice-cold PBS using a 1 ml pipette tip (the tip can be cut to widen the bore in order to avoid clogging the tip).
3. Centrifuge the 15 ml conical tubes at 500 x g for 5 minutes at 4°C.
4. Aspirate PBS from centrifuged tube, and add 1 ml ATAC Lysis Buffer.
5. Using a 1 ml pipette tip with a widened bore, transfer each sample to a 1 ml dounce homogenizer and dounce slowly for 30 strokes using a tight-fitting pestle (for example, small-clearance, type B).
6. Filter each homogenized sample through a 40 µm mesh strainer and collect in a fresh 1.5 ml microcentrifuge tube. Immediately after filtration, take a 10 µl aliquot for cell counting.
7. Count the cells in each aliquot using Trypan Blue cell-viability staining (stain in a 1:1 ratio of cell volume:0.4% Trypan Blue). Only nuclei stained blue by Trypan Blue should be counted.
8. Invert cell samples gently to mix, then aliquot 50,000 - 100,000 cells into a new tube.
9. Centrifuge the new cell aliquots at 500 x g at 4°C for 5 minutes. During this time, prepare the Tagmentation Master Mix.

Tagmentation Master Mix (per sample)

Reagent	Volume
2X Tagmentation Buffer	25 µl
10X PBS	2 µl
1.0% Digitonin	0.5 µl
10% Tween 20	0.5 µl
H_2O	12 µl
Assembled Transposomes	10 µl

10. Aspirate supernatant and proceed immediately to the Tagmentation Reaction and Purification steps.

Cell Sample Preparation

50,000 to 100,000 fresh cells or cryopreserved frozen cells can be used. Cells must be of high quality to preserve viability. Cryopreserved cells should be cryopreserved in a controlled rate freeze with media formulated to protect against the ice crystal formation and subsequent cell damage. (Note, tissue should be flash frozen at -80°C)

Take SPRI beads out of 4°C and warm to room temperature by simply placing the vial of SPRI beads on the benchtop.

1. Count cells and aliquot 50,000 to 100,000 cells into a fresh 1.5 ml centrifuge tube for each sample.
2. Centrifuge the cells at 500 x g for 5 minute at 4°C. Orient the tubes such that the pellet will be where you expect it to be. If there is no visible pellet, spin an additional 5 minutes at 1,000 x g at 4°C.
3. Gently remove supernatant by pipetting and add 100 µl of ice-cold PBS. Do not resuspend or disturb pellet. Spin once more at 500 x g for 5 minutes at 4°C.
4. Remove supernatant very carefully, ensuring to not disturb the cell pellet. By pipetting, thoroughtly resuspend the cell pellet in 100 µl ice-cold ATAC Lysis Buffer.
5. Transfer the resuspended cell pellet to a PCR tube on ice. Immediately spin down at 500 x g for 10 minutes at 4°C. During this time, prepare the Tagmentation Master Mix.

   Tagmentation Master Mix (per sample)

Reagent	Volume
2X Tagmentation Buffer	25 µl
10X PBS	2 µl
1.0% Digitonin	0.5 µl
10% Tween 20	0.5 µl
H_2O	12 µl
Assembled Transposomes	10 µl

6. After the spin, remove the supernatant very carefully, ensuring the cell pellet is not disturbed (this is a critical step; take extra care to remove the ATAC Lysis Buffer but not lose nuclei). Immediately continue to the Tagmentation Reaction and Purification steps.

Tagmentation Reaction and Purification

1. Add 50 µl of Tagmentation Master Mix to each sample (does not have to be on ice). Gently pipette to resuspend nuclei in the Tagmentation Master Mix with a multi-channel pipettor.
2. Incubate the tagmentation reaction at 37°C for 30 minutes in a thermomixer (or similar device) set at 800 rpm.
3. Immediately following the tagmentation reaction, transfer each sample to a clean 1.5 ml microcentrifuge tube.
4. Add 250 µl DNA Purification Binding Buffer and 5 µl 3 M sodium acetate to each sample.
5. If the color of the sample is anything other than bright yellow, add additional 3 M sodium acetate in 5 µl increments until the proper color is achieved. Please see the figure below.

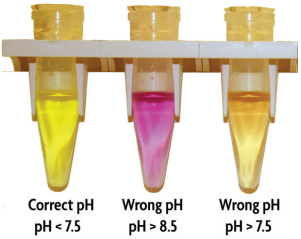

Figure 1: Solution color as a function of pH.

The DNA Purification Binding Buffer has a pH indicator dye, so that the pH of the solution can easily be determined. Only apply the sample to the column when the solution is bright yellow (tube on the left), indicating a pH under 7.5. DNA will not bind to the column if the pH is higher than 7.5. Note: a full-color PDF of this manual can be downloaded from the Active Motif website, where you can see this figure in color.

6. Mix each sample by pipetting.
7. For each sample, place a labeled DNA purification column into a collection tube.
8 Transfer each sample to its corresponding column, close the cap, and centrifuge at 17,000 x g (14,000 rpm) for 1 minute.
9. Discard the flow-through and return the collection tube to the column.

   Note: 100% ethanol must be added to the Wash Buffer before the first use, to a final concentration of 80%.
10. Add 750 µl of Wash Buffer (see note above in step 9 the Wash Buffer should have a final concentration of 80% ethanol) to the column and cap the column.
11. Centrifuge at 17,000 x g for 1 minute.
12. Discard the flow-through and return the collection tube to the column.

13. With the column cap open, centrifuge at 17,000 x g for 2 minutes to remove residual Wash Buffer from the column.
14. Transfer each column to a new microcentrifuge tube.
15. Add 35 µl of DNA Purification Elution Buffer to the center of the column matrix, cap the column, and incubate for 1 minute at room temperature.
16. Centrifuge at 17,000 x g for 1 minute.
17. Discard column. DNA purification is complete.
18. Purified DNA can be stored at -20°C, or you may proceed directly with the PCR Amplification of Tagmented DNA steps that follow.

PCR Amplification of Tagmented DNA

Note: If a kit such as the KAPA Real-Time Library Amplification Kit is used, an initial 72°C extension step is essential.

1. Set up the PCR reactions by adding the components in the order shown below. If libraries are to be multiplexed for sequencing on the same flow cell, ensure that a unique i5 and/or i7 index combination is used for each.

   Each sample will require a combination of one i7 Indexed Primer and one i5 Indexed Primer in the PCR amplification reaction. There are 4 x 4 = 16 unique combinations of i7/i5 primers for a total of 16 samples that can be multiplexed. These Indexed Primers are based on Illumina's Nextera adapters.

   Per reaction:

Use one i7 Indexed Primer	**And use one i5 Indexed Primer**
i7 Indexed Primer 1 = i7 N701	i5 Indexed Primer 1 = i5 N501
i7 Indexed Primer 2 = i7 N702	i5 Indexed Primer 2 = i5 N502
i7 Indexed Primer 3 = i7 N703	i5 Indexed Primer 3 = i5 N503
i7 Indexed Primer 4 = i7 N704	i5 Indexed Primer 4 = i5 N504

Reagent	Volume
Tagmented DNA	33.5 µl
i7 Indexed Primer (25 µM)	2.5 µl
i5 Indexed Primer (25 µM)	2.5 µl
dNTPs (10 mM)	1 µl
5X Q5 Reaction Buffer	10 µl
Q5 Polymerase (2 U/µl)	0.5 µl

2. Perform PCR using the following program on a thermal cycler (with a heated lid):

   72°C 5 minutes

   98°C for 30 seconds

   10 cycles of: 98°C for 10 seconds, 63°C for 30 seconds, 72°C for 1 minute

   Hold at 10°C.

3. Perform SPRI clean-up with 60 µl SPRI bead solution (1.2X the sample volume), eluting in 20 µl DNA Purification Elution Buffer. Have 400 µl of freshly-prepared 80% ethanol ready per sample:

   a. Add 60 µl well-mixed, room temperature SPRI Beads to each sample.

   b. Vortex briefly to mix and incubate for 5 minutes at room temperature to allow beads to bind.

   c. Apply magnet to collect beads.

   d. Once the solution is clear, aspirate the supernatant.

   e. With the magnet still applied to the sample, add 180 µl 80% ethanol to each sample without mixing.

   f. Incubate for 30 seconds at room temperature.

   g. Aspirate the supernatant.

   h. Repeat steps e through g for a second ethanol wash.

   i. Allow tubes to sit at room temperature so that residual ethanol can evaporate. Once the beads transition from shiny to matte (2-5 minutes), proceed to the next step.

   j. With the tubes separated from the magnet, add 20 µl DNA Purfication Elution Buffer.

   k. Cap tubes and vortex to mix.

   l. Incubate samples for 5 minutes at room temperature.

   m. Apply magnet to collect beads.

   n. Once the solution is clear, transfer each supernatant containing the eluted DNA to a fresh tube.

4. At this stage, libraries are ready for quantification and sequencing. Use a library quantification kit for next-generation sequencing to quantify the library (e.g. Kapa Biosystems, Catalog No. KR0405). PCR amplified libraries can also be analyzed to assess size distribution with a Bioanalyzer, TapeStation, or similar instrument to assess size distribution.

Index Primers and Sample Sheet Information

Index 1 (i7) Primers

CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG

Index 2 (i5) Primers

AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC

i7 Index	i7 Sequence	Sample Sheet
N701	TCGCCTTA	TAAGGCGA
N702	CTAGTACG	CGTACTAG
N703	TTCTGCCT	AGGCAGAA
N704	GCTCAGGA	TCCTGAGC

i5 Index	i5 Sequence	Sample Sheet (NovaSeq v1.0 Reagent Kits, MiSeq, HiSeq 2000/2500)
N501	TAGATCGC	TAGATCGC
N502	CTCTCTAT	CTCTCTAT
N503	TATCCTCT	TATCCTCT
N504	AGAGTAGA	AGAGTAGA

i5 Index	i5 Sequence	Sample Sheet (NovaSeq v1.5 Reagent Kits iSeq, MiniSeq, NextSeq, HiSeq 3000/4000)
N501	TAGATCGC	GCGATCTA
N502	CTCTCTAT	ATAGAGAG
N503	TATCCTCT	AGAGGATA
N504	AGAGTAGA	TCTACTCT

Sequence for Read 1 and Read 2 adapter trimming: CTGTCTCTTATACACATCT.

References

1. Buenrostro, J. D., *et al.* (2013) *Nat. Methods* 10: 1213-1218.
2. Adley, A., *et al.* (2010) *Genome Biol.* 11.
3. Corces, M. R. *et al.* (2017) *Nat. Methods* 14: 959-962.

Section B. Troubleshooting Guide

Problem/question	Possible cause	Recommendation
High background in sequencing data	Cell viability may be the issue. Apoptotic cells release unprotected DNA that is much more accessible to Tn5 than DNA in compacted chromatin, leading to high background signal.	Optional treatment with DNase I can improve results. This treatment is only an option of cells that are viable and able to exclude the enzyme[3].
No library produced	Sample loss is possible throughout the protocol, especially when working with small numbers of cells as the pellet may be difficult or impossible to see.	Be sure to orient tubes so you know where the cell pellet will be, and pipette from the side of the tube that is opposite from the pellet in all centrifugation steps except for the removal of lysis buffer where a few microliters of supernatant can be left behind.
	Incompatible amplification program used.	An initial 72°C extension step before denaturation is essential for ATAC-Seq libraries because the 5' ends of adapters' non-transferred strands are not ligated to insert DNA by the enzyme. The extension is therefore required in order to produce the anchor site for the index primers. There is no real recovery from this, unfortunately.

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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