Cell Biologics™ ATAC-Seq kit

(Cat. No. CB6936)

Instruction Manual

Description

The Cell Biologics™ ATAC-seq kit is a complete set of optimized reagents to generate high quality indexed DNA libraries for ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) on Illumina® sequencing platform.

Cell Biologics™ ATAC-Seq Kit Includes:

The volumes provided are sufficient for preparation of up to 24 reactions/samples. (All reagents should be stored at –20°C).

1. Transposome
2. 2x Reaction Buffer
3. Nuclease Free H2O
4. High-Fidelity 2x PCR Master Mix
5. Ad1.noMX (Oligo 1)
6. Ad2.1 – Ad2.24 (24-Indexed Oligo 2)

Required Materials Not Included:

1. Magnetic Rack (Thermofisher, cat # CS15000 or equivalent)
2. Qiagen MinElute PCR Purification Kit (cat # 28004)
3. Agencourt® AMPure® XP Beads (Beckman Coulter, Inc. cat #A63881)
4. Tris-HCl, pH 7.5–8.0
5. PBS
6. IGEPAL CA-630 (Sigma-Aldrich, cat # I8896)
7. NaCl
8. MgCl2
9. 80% Ethanol (freshly prepared)
10. NEBNext® Library Quant Kit for Illumina (NEB, cat#E7630)

I. Cell Preparation

1. Harvest fresh cells (no fixation), protocol to be defined by the user.
2. Spin down 50,000 cells at 500 ×g for 5 min, 4°C.
3. Wash once with 500 µL of cold 1x PBS buffer. Spin down at 500 ×g for 5 min, 4°C.
4. Gently pipette to resuspend the cell pellet in 450 µL of cold hypotonic buffer (10 mM Tris-HCl,
5. pH 7.4, 10 mM NaCl, 3 mM MgCl2). Immediately add 50 uL 1% IGEPAL CA-630 (0.1% final),
6. invert to mix (do not vortex!)
7. Incubate on ice for 10 to 15 min (Or monitoring lysis by taking 5 µL samples, mixing with 5 µL
8. 0.4% trypan blue prepared in PBS and observing % lysed cells. Target is >85% blue (lysed) cells).
9. Spin down at 500 ×g for 10 min, 4°C.
10. Discard the supernatant, and immediately continue to transposition reaction.

II. Transposition Reaction and Purification

1. Make sure the cell pellet is set on ice.
2. To make the transposition reaction mix, combine the following (50 µl Total):

Component	Volume
2x Reaction Buffer	25 µL
Transposome	2.5 µL
Nuclease Free H2O	22.5 µL

3. Gently pipette to resuspend nuclei in the transposition reaction mix.
4. Incubate the transposition reaction at 37°C for 60 min.
5. Immediately following transposition, purify using Qiagen MinElute kit (Qiagen, #28004).
6. Elute transposed DNA in 15 µL Elution Buffer (10mM Tris buffer, pH 8).
7. Purified DNA can be stored at -20°C.

III. PCR Amplification (Library Generation)

A. To amplify transposed DNA fragments, combine the following in a PCR tube:

- 10 µL Transposed DNA
- 10 µL Nuclease Free H2O
- 2.5 µL Ad1.noMX(Oligo 1)
- 2.5 µL Ad2. *_[Barcode] (Oligo 2, **** choose one from Ad2.1 to Ad2.24***)
- 25 µL High-Fidelity 2x PCR Master Mix
- 50 µL Total

B. Run the above 50 µL PCR reaction on a thermocycler. Cycle as follows:

- 98°C, 30 sec
- 98°C, 10 sec
- 63°C, 30 sec
- 72°C, 1 min
- Repeat steps 2-4, 10 times
- Hold at 4°C

IV. Library Purification

- Purify the amplified the libraries using AMpure XP beads as follows:
  (Warm AMPure XP beads to room temperature, and vortex to resuspend.)

A. For single left-sided bead purification (to remove primer dimers):

1. Transfer each PCR sample to an Eppendorf tube, add 1.8X volume (90 µl) AMPure XP beads, pipet up and down 10 times to mix thoroughly.
2. Incubate at room temperature for 10 minutes.
3. Place tubes in magnetic rack for 5 minutes.
4. Discard supernatant.
5. Wash beads with 200 µl 80% EtOH (freshly made), pipette EtOH over beads 10 times, and then discard EtOH.
6. Leave tube on magnetic rack with cap open for 10 minutes.
7. Ensure all EtOH is removed.
8. Resuspend beads in 20 µl nuclease-free H2O, pipet up and down 10 times to mix thoroughly.
9. Place tube in magnetic rack for 1-5 minutes.
10. Transfer supernatant to new Eppendorf tube.

B. For double-sided bead purification (to remove primer dimers and large >1,000 bp fragments):

1. **Transfer each PCR sample to an Eppendorf tube, add 0.5X volume (25 µl) AMPure XP beads,** pipet up and down 10x to mix thoroughly.
2. Incubate at room temperature for 10 minutes.
3. Place tubes in magnetic rack for 5 minutes.
4. Transfer supernatant to new epi tube.
5. Add 1.3X original volume (65 µl) AMPure XP beads, pipet up and down 10 times to mix thoroughly. (This results in a final 1.8X bead buffer : sample ratio.)
6. Incubate at room temperature for 10 minutes.
7. Place epi tubes in magnetic rack for 5 minutes.
8. Discard supernatant.
9. Wash beads with 200 µl 80% EtOH (freshly made), pipet EtOH over beads 10 times, then discard EtOH.
10. Leave tube on magnetic rack with cap open for 10 minutes.
11. Ensure all EtOH is removed.
12. Resuspend beads in 20 µl nuclease-free H2O, pipet up and down 10 times to mix thoroughly.
13. Place tube in magnetic rack for 1-5 minutes.
14. Transfer supernatant to new Eppendorf tube.
15. Store purified libraries at -20°C

V. Assessing Library Quality (Optional)

- Use qPCR based methods to quantify purified ATAC-seq libraries with a NEBNext® Library Quant Kit for Illumina® (NEB, #E7630). Bioanalyzer and Qubit, can give misleading and inaccurate results due to the large distribution of insert sizes.

VI. Sequencing

- Paired-end sequencing on an Illumina platform (HiSeq 2500 / HiSeq 4000, et al) with >50 million reads per sample minimum is recommended.

VII . ATAC-seq Oligo for PCR

Ad1_noMX:	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
Ad2.1_TAAGGCGA	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Ad2.4_TCCTGAGC	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
Ad2.5_GGACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
Ad2.6_TAGGCATG	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8_CAGAGAGG	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
Ad2.9_GCTACGCT	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
Ad2.10_CGAGGCTG	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
Ad2.11_AAGAGGCA	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
Ad2.12_GTAGAGGA	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
Ad2.13_GTCGTGAT	CAAGCAGAAGACGGCATACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
Ad2.14_ACCACTGT	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT
Ad2.15_TGGATCTG	CAAGCAGAAGACGGCATACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGT
Ad2.16_CCGTTTGT	CAAGCAGAAGACGGCATACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGT
Ad2.17_TGCTGGGT	CAAGCAGAAGACGGCATACGAGATACCCAGCAGTCTCGTGGGCTCGGAGATGT
Ad2.18_GAGGGGTT	CAAGCAGAAGACGGCATACGAGATAACCCCTCGTCTCGTGGGCTCGGAGATGT
Ad2.19_AGGTTGGG	CAAGCAGAAGACGGCATACGAGATCCCAACCTGTCTCGTGGGCTCGGAGATGT
Ad2.20_GTGTGGTG	CAAGCAGAAGACGGCATACGAGATCACCACACGTCTCGTGGGCTCGGAGATGT
Ad2.21_TGGGTTTC	CAAGCAGAAGACGGCATACGAGATGAAACCCAGTCTCGTGGGCTCGGAGATGT
Ad2.22_TGGTCACA	CAAGCAGAAGACGGCATACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGT
Ad2.23_TTGACCCT	CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGT
Ad2.24_CCACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTGGGTCTCGTGGGCTCGGAGATGT