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Pre-indexed Assembled Tn5 Transposomes

Catalog No. 53152

(Version A4)

Revision	Date	Description of Change
A2	December, 2023	Added "High Fidelity DNA polymerase" to the Additional Materials Required section on page 3
A3	August, 2024	Corrected error in table on page 2
A4	October, 2024	Updated Pre-indexed Assembled Tn5 Transposomes ATAC-Seq Assay Protocol Step 11

Contents

Background

Pre-indexed Assembled Tn5 Transposomes are provided in a breakaway 96-well plate format to enable higher throughput ATAC-Seq assays. By having a unique i7 & i5 Indexed Primer combination in each well, it is easy to perform multiple ATAC-Seq reactions without having to pipette primers separately and keep track of the combinations. The ATAC-Seq Buffer Set (Cat. No. 53153) is a convenient set of buffers to use for 96 ATAC-Seq reactions with the Pre-indexed Assembled Tn5 Transposomes.

How does the ATAC-Seq Assay work with the Pre-indexed Assembled Tn5 Transposomes? Simply add 50,000 to 100,000 prepared cells per well, and incubate with Tagmentation Master Mix for 30 minutes. Then, stop the reaction and collect tagmented DNA using SPRI beads and purify the DNA and PCR amplify. The libraries are then ready for sequencing on an Illumina® Sequencing System. P5 and P7 Primers are included with the Pre-indexed Assembled Tn5 Transposomes for post tagmentation PCR amplification.

Product	Format	Catalog No.
Pre-indexed Assembled Tn5 Transposomes	1 x 96 rxns	53152

Kit Components

Reagents	Quantity	Storage
Pre-indexed Assembled Tn5 Transposomes	1 x 96 rxns	-20°C
P5 Primer (25 µM)	250 µL	-20°C
P7 Primer (25 µM)	250 µL	-20°C

Note: The Pre-indexed Assembled Tn5 Transposomes must be stored at -20°C. Improper storage results in poor ATAC-Seq assay results. The Pre-indexed Assembled Tn5 Transposomes are formulated in glycerol and are stable through quick freeze-thaw cycles when breaking away the wells for use in an assay. Be sure to store remaining wells at -20°C.

This product is guaranteed for six months from date of receipt when components are stored properly.

Well Location		1	2	3	4	5	6	7	8	9	10	11	12
	Index	N505	N506	N507	N508	N510	N511	N513	N515	N516	N517	N518	N520
A	N701	N701, N505	N701, N506	N701, N507	N701, N508	N701, N510	N701, N511	N701, N513	N701, N515	N701, N516	N701, N517	N701, N518	N701, N520
B	N702	N702, N505	N702, N506	N702, N507	N702, N508	N702, N510	N702, N511	N702, N513	N702, N515	N702, N516	N702, N517	N702, N518	N702, N520
C	N703	N703, N505	N703, N506	N703, N507	N703, N508	N703, N510	N703, N511	N703, N513	N703, N515	N709*, N516	N703, N517	N703, N518	N703, N520
D	N704	N704, N505	N704, N506	N709*, N507	N704, N508	N704, N510	N704, N511	N704, N513	N704, N515	N704, N516	N704, N517	N704, N518	N704, N520
E	N705	N705, N505	N705, N506	N705, N507	N705, N508	N705, N510	N705, N511	N705, N513	N705, N515	N705, N516	N705, N517	N705, N518	N705, N520
F	N706	N706, N505	N706, N506	N706, N507	N706, N508	N706, N510	N706, N511	N706, N513	N706, N515	N706, N516	N706, N517	N706, N518	N706, N520
G	N707	N707, N505	N707, N506	N707, N507	N707, N508	N707, N510	N709*, N511	N707, N513	N707, N515	N707, N516	N707, N517	N709*, N518	N707, N520
H	N708	N708, N505	N708, N506	N708, N507	N708, N508	N708, N510	N708, N511	N708, N513	N708, N515	N708, N516	N708, N517	N708, N518	N708, N520

Figure: Pre-indexed Assembled Tn5 Transposomes Indexed Primer Plate Map

Each well contains 4 µL of 8 µM Pre-indexed Assembled Tn5 Transposomes. This concentration is a combination of the indexed primer pairs and Tn5 (8 µM Tn5 + 4 µM oligo A + 4 µM oligo B). The Pre-indexed Assembled Tn5 Transposomes have been validated in ATAC-Seq using 50,000 cells. All i5 and i7 index combinations were tested to ensure efficiency in a biologically relevant tagmentation reaction. i5 and i7 index combinations that were identified to produce less sequencing reads were replaced with index N709 and normal sequencing reads resulted after re-testing to confirm they yield optimal results. Wells where N709 replaced the index in use for other wells in that row are highlighted with red N709* in the plate map above to easily see the changes in the logical layout of the plate.

Protocol

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.

Additional Materials Required

- Micropipette and appropriate tips
- ATAC-seq Buffer Set (Cat. No. 53153) or 1% Digitonin, 2X Tagmentation Buffer, and ATAC Lysis Buffer
- 10X PBS
- 10% Tween20
- 50,000-100,000 nuclei
- Nuclease-free water
- Thermal shaker for PCR tubes
- DNA purification column clean up or 0.5M EDTA, 10% SDS and SPRI beads
- Thermal cycler
- High Fidelity DNA polymerase

Pre-indexed Assembled Tn5 Transposomes ATAC-Seq Assay Protocol

1. Count cells and aliquot 50,000 to 100,000 cells into a fresh 1.5 ml centrifuge tube, or 96-well plate for each sample.
2. Centrifuge the cells at 500 x *g* for 5 minute at 4°C. 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, thoroughly resuspend the cell pellet in 100 µl ice-cold ATAC Lysis Buffer.
5. Immediately spin down at 500 x *g* for 10 minutes at 4°C. During this time, prepare the Tagmentation Master Mix.
6. Prepare the following Tagmentation Master Mix and keep on ice

Ingredient	Per Sample Volume
2X Tagmentation Buffer	25 µL
10X PBS	2 µL
1% Digitonin	0.5 µL
10% Tween20	0.5 µL
Nuclease-free Water	18 µL
Total	**46 µL**

7. 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).
8. Immediately resuspend each nuclei sample in 46 µL of the Tagmentation Master Mix prepared in the previous step. Keep on ice.
9. Retrieve 96-well Pre-indexed Assembled Tn5 Transposomes plate from -20°C and tear away the number of strips or wells desired. Promptly freeze any unused wells at -20°C.
10. Quickly, but gently, spin down the strips or plate in a centrifuge to collect all liquid at the bottom of the well.
11. Working with the plate or strip wells containing the Pre-indexed Assembled Tn5 Transposomes in a cold block or on ice, remove the strip caps and add resuspended nuclei from step 8 directly to the well. Be sure to keep track of the orientation of the caps so the cap returns to the correct tube.
12. Re-cap the wells, gently vortex, and quick spin.
13. Incubate Pre-indexed Assembled Tn5 Transposomes plate or strip wells containing nuclei and Tn5 at 37°C with shaking at 800 rpm for 30 minutes.

14. After the incubation, stop the tagmentation reaction using method A or B:

    A. EDTA and SPRI Beads

        i. Add 1.8 µL of 0.5M EDTA and 0.5 µL of 10% SDS directly to the tagmentation well

        ii. Re-cap, vortex to mix, quick spin, and incubate the sample at 55°C for 3 minutes

        iii. Collect tagmented DNA using desired SPRI bead ratio (1.2X ratio is recommended)

    B. Purify the sample on a DNA purification column following the column specific protocol.

At this point, purified, tagmented DNA fragments can be prepared by PCR amplified for next generation sequencing on an Illumina® Sequencing System. The PCR amplification primers included are supplied as 25 µM in TE buffer. Using the primers at a final concentration of 1.25 µM in the PCR reaction is recommended (2.5 µL of each primer per 50 µL reaction).

The following PCR protocol is recommended:

Note: The number of cycles is based on starting with 50,000 – 100,000 nuclei. This step will need to be optimized if more or less starting material is used.

72°C for 5 minutes
98°C for 30 seconds
8-10 cycles of:
    98°C for 10 seconds
    63°C for 30 seconds
    72°C for 1 minute
Hold at 10°C

Note: If you plan to sequence your samples, we recommend purifying the amplified DNA using 1.2x SPRI beads relative to the sample volume.

For index primer and sample sheet information, visit the Pre-indexed Assembled Tn5 Transposomes web page at activemotif.com

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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