CUT&Tag-IT™ Assay Kit, Anti-Mouse Manual

(version A5)

Catalog No. 53165 (16 rxns)

US Pat. No. 10,689,643, EP Pat. No. 2999784
and related patents and applications

Revision	Date	Description of Change
A3	January, 2024	Tagmentation Buffer storage temp changed from -20°C to 4°C or -20°C
A4	April, 2024	Added notes to advise on optimal cell numbers
A5	March, 2025	Added images and guidelines for using Concanavalin A Beads

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Overview

Cleavage Under Targets and Tagmentation (CUT&Tag) is a method to investigate genomic localization of histone modifications and some transcription factors that reveals interactions between proteins and DNA or identifies DNA binding sites for proteins of interest1.

Unlike MNase-Seq or ATAC-Seq methods that target open chromatin and are therefore dependent on chromatin accessibility, CUT&Tag utilizes an antibody-based enzyme tethering strategy to target specific histone modifications or proteins to reveal chromatin-binding information that is specific to those sites or proteins of interest.

CUT&Tag is based on the same principles as ChIP-Seq, but with several changes to the protocol that are advantageous in certain situations. Instead of the sonication of fixed chromatin and immunoprecipitation steps performed in ChIP-Seq protocols, in CUT&Tag, fresh (not frozen) unfixed cells are bound to concanavalin A beads and the antibody incubation is performed with cells in their native state. Directly following antibody binding, the chromatin is sheared and NGS libraries are prepared in a single step by tagmentation using the protein A-Tn5 (pA-Tn5) transposome enzyme that has been pre-loaded with sequencing adapters.

The CUT&Tag-IT™ Assay Kit is optimized for 50,000 to 500,000 cells per reaction, although 200,000 cells are recommended. This Kit provides optimized reagents and protocol to produce 16 unique sequencing-ready Illumina®-compatible libraries.

CUT&Tag-IT™ Assay Kit Advantages

- Compatible with as few as 5,000 cells
- Low background signal enables lower sequencing depth
- No artifacts caused by formaldehyde crosslinking

product	format	catalog no.
CUT&Tag-IT™ Assay Kit, Anti-Mouse	16 reactions	53165

Kit Components and Storage

The kit contains sufficient reagents for 16 CUT&Tag Assay reactions for use with a mouse primary antibody to target or mark of interest. 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.

CUT&Tag Assay Kit Reagents

Reagents	Quantity	Storage
5% Digitonin	610 µL	-20°C
Concanavalin A Beads	320 µL	4°C
CUT&Tag-IT™ Assembled pA-Tn5 Transposomes	16 µL	-20°C
Tagmentation Buffer	2 mL	4°C or -20°C
1X Binding Buffer	55 mL	4°C
1X Wash Buffer	40 mL	4°C
Dig-Wash Buffer	50 mL	4°C
Antibody Buffer	800 µL	4°C
Dig-300 Buffer	50 mL	4°C
Rabbit Anti-Mouse Antibody	16 µL	-20°C
Protease Inhibitor Cocktail	1.42 mL	-20°C
0.5M EDTA	68 µL	RT
10% SDS	20 µL	RT
10 µg/µL Proteinase K	18 µL	-20°C
DNA Purification Columns	16 columns	RT
DNA Purification Binding Buffer	10 mL	RT
DNA Purification Wash Buffer	12 mL	RT
DNA Purification Elution Buffer	5 mL	RT
3M Sodium Acetate	128 µL	RT
10 mM DNTPs	16 µL	-20°C
5X Q5 Buffer	160 µL	-20°C
Q5 High Fidelity DNA Polymerase (2U/µL)	8 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
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
SPRI Beads	880 µL	4°C

Additional Materials Required

- Molecular grade nuclease free water
- 100% Ethanol
- 80% Ethanol
- Magnetic Rack for 1.5/2 mL tubes
- Magnetic Rack for 200 µL 8-well PCR strip tubes
- Nutator or orbital rotating mixer
- Vortexer
- Thermal cycler
- Illumina® NextSeq® Sequencer
- 1.5 mL Low-bind Microcentrifuge tubes
- 2 mL Low-bind Microcentrifuge tubes
- Microcentrifuge for 8-well PCR strip tubes with caps, or for 0.2 and 1.5 mL tubes
- Multichannel pipette (20 - 200 µL)
- Filter tips for pipettes

This Kit is optimized for 50,000 to 500,000 cells, although 200,000 cells are recommended. This Kit is for use with a mouse primary antibody to the histone mark of interest.

NOTE: This protocol is optimized for non-adherent cells. If adherent cells are to be used, avoid using Trypsin or cell detachment solutions that digest cell membrane proteins. Cell membrane proteins are necessary to bind the cells to the Concanavalin A Beads in Section B of the assay. To lift adherent cells from growth plates, use an enzyme-free dissociation method such as scraping with a rubber policeman.

Section A. Prepare Fresh Cells (30 minutes)

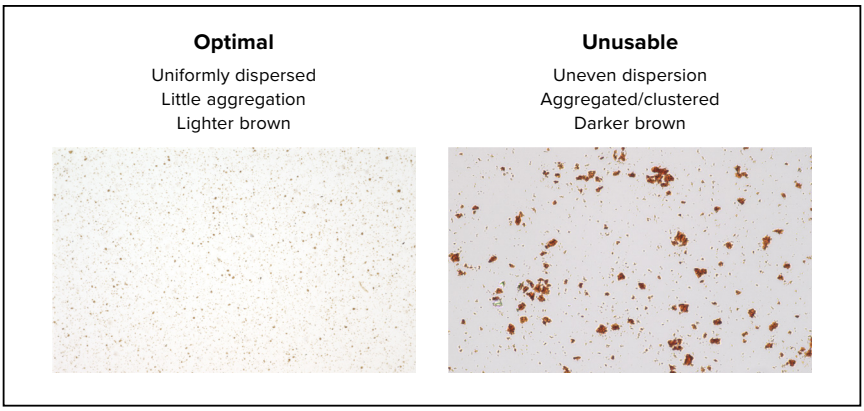
NOTE: Before using 1X Wash Buffer in Step 3 below, you must add Protease Inhibitor Cocktail to the 1X Wash Buffer. Add 10 µL Protease Inhibitor Cocktail for every 1 mL of 1X Wash Buffer. 2.5 mL total are used per cell sample. Keep on ice. Wash Buffer can be stored at 4°C for up to 1 week once Protease Inhibitor Cocktail has been added.

1. Harvest fresh culture(s) at room temperature and count cells. Place the desired amount of cells into a 1.5 mL microcentrifuge tube.
2. Centrifuge for three minutes at 600 X g at room temperature, then remove supernatant.
3. Resuspend in 1 mL 1 X Wash Buffer at room temperature, then centrifuge for another three minutes at 600 X g at room temperature, and remove supernatant.
4. Resuspend in 1.5 mL 1 X Wash Buffer and transfer to a 2 mL microcentrifuge tube. Place tube with resuspended pellet on ice while the Concanavalin A beads are prepared in the next steps.

Section B. Bind Cells to Concanavalin A Beads (30 minutes)

NOTE: When preparing beads, cells, or nuclei, the buffers are kept on ice but the samples are handled on the bench at room temperature for convenience.

Check the Concanavalin A beads under a microscope before starting the experiment. If the beads are aggregated or clustered do not proceed with the experiment and contact technical services.



NOTE: This Kit is optimized for 50,000 to 500,000 cells. Best results have been obtained with 200,000 cells. 500,000 cells can become viscous in the steps below (Bind Cells to Concanavalin A Beads, Steps 5-9) and DNA purification.

5. Gently resuspend and withdraw enough of the Concanavalin A Bead slurry such that there will be 20 µL per each sample. Use 20 µL for up to 500,000 cells. The following steps are written for one sample of 500,000 cells. Do not adjust the volumes for lower cell numbers.
6. Into a 2 mL tube, transfer 20 µL Concanavalin A bead slurry into 1.6 mL 1 X Binding Buffer and mix by pipetting. Place the tube on a magnetic stand to clear (30 seconds to 2 minutes).
7. Withdraw the liquid completely and remove the tube from the magnetic stand. Add 1.5 mL 1 X Binding Buffer, mix by pipetting, and collect any liquid from the sides or cap with a quick pulse spin in a microcentrifuge.
8. Place tube on magnetic stand to clear (30 seconds to 2 minutes), and remove liquid by pipette. Resuspend in 20 µL 1 X Binding Buffer (20 µL per 500,000 cells per reaction) and keep the tube containing prepared Concanavalin A Beads at room temperature until the fresh cells are ready.
9. Slowly add the bead slurry from Step 8 and invert tube to mix. Place the tube containing the cells and Beads on an end-over-end rotator for 10 minutes.

Section C. Bind Primary Antibody (2 hours to overnight)

NOTE: Before using Antibody Buffer in Step 11 below, you must add Protease Inhibitor Cocktail and Digitonin. For every 1 mL of buffer to be used, add 10 µL of Protease Inhibitor Cocktail and 10 µL of 5% Digitonin. Keep on ice. Antibody Buffer can be kept at 4°C for up to 2 days once Protease Inhibitor Cocktail and Digitonin have been added. For each reaction, you will need 50 µL supplemented with 0.5 µL Protease Inhibitor Cocktail and 0.5 µL of 5% Digitonin.

10. After a quick spin (<100 X g) to remove liquid from the cap, place the tubes on a magnetic

stand to clear (30 seconds to 2 minutes), and remove the liquid via a pipette.

11. Resuspend cells in a volume of ice-cold Antibody Buffer such that each sample is in a 50 µL volume, not to exceed 500,000 cells per 50 µL. Gently vortex. Place the tube on ice.
12. Add 1 µL (or at least 1 µg) undiluted primary antibody to each sample, and gently vortex or pipette to mix.

    IMPORTANT: Use a mouse primary antibody. We recommend using a 1:50 to 1:100 dilution or the antibody manufacturer's recommendation for the dilution used in applications such as immunofluorescence. Histone H3K27me3 (Cat. No. 61018) is a common mark that can be a positive control.

13. Incubate 2 hours to overnight at 4°C with orbital mixing, make certain liquid stays together in the bottom of the tube so that it all mixes together.

Section D. Bind Secondary Antibody (60 minutes)

NOTE: Before using Dig-Wash Buffer in Step 15 below, you must add Protease Inhibitor Cocktail and Digitonin. For every 1 mL of buffer, add 10 µL Protease Inhibitor Cocktail and 10 µL of 5% Digitonin. 3.1 mL total are used per sample. Keep on ice. Dig-Wash Buffer can be kept at 4°C for up to 2 days once Protease Inhibitor Cocktail and Digitonin have been added.

14. After a quick spin (<100 X g), place each tube on a magnetic stand to clear (30 seconds to 2 minutes), and then remove the liquid via a pipette.
15. Dilute the Rabbit Anti-Mouse secondary antibody 1:100 in Dig-Wash Buffer. 100 µL of the diluted secondary are needed per reaction. Add 100 µL of this diluted secondary antibody to each sample while gently vortexing to dislodge the beads from the sides of the tube.
16. Place the tubes on an orbital rotator at room temperature for 60 minutes.
17. After a quick spin, place the tubes on a magnetic stand to clear (30 seconds to 2 minutes), then remove the liquid via a pipette.
18. Add 1 mL Dig-Wash Buffer to each reaction. Gently vortex or pipette to dislodge any beads that have aggregated together.
19. Repeat Step 18 twice, for a total of three washes.

Section E. Bind CUT&Tag-IT™ Assembled pA-Tn5 Transposomes (60 minutes)

NOTE: Before using Dig-300 Buffer in Step 20 below, you must add Protease Inhibitor Cocktail and Digitonin. For every 1 mL of buffer, add 10 µL Protease Inhibitor Cocktail and 2 µL of 5% Digitonin. 3.1 mL total are used per sample. Keep on ice. Dig-300 Buffer can be kept at 4°C for up to 2 days once Protease Inhibitor Cocktail and Digitonin have been added.

20. Dilute the CUT&Tag-IT™ Assembled pA-Tn5 Transposomes with Dig-300 Buffer to a final concentration of 1:100. 100 µL will be needed per reaction. For example, for one reaction, add 1 µL of the CUT&Tag-IT™ Assembled pA-Tn5 Transposomes to 100 µL of Dig-300 Buffer.
21. After a quick spin (<100 X g), place each tube containing the immunoprecipitated samples on a magnetic stand to clear (30 seconds to 2 minutes), and then remove the liquid via a pipette.
22. Add 100 µL of the diluted CUT&Tag-IT™ Assembled pA-Tn5 Transposomes from Step 20 while gently pipetting to make sure it mixes together.
23. Incubate the reactions at room temperature on an orbital rotator for 60 minutes.
24. After a quick spin (<100 X g), place the tubes on a magnetic stand to clear (30 seconds to 2 minutes) and remove the liquid with a pipette.
25. Add 1 mL Dig-300 Buffer. Gently vortex or pipette to dislodge the beads.
26. Repeat Step 25 twice for a total of three washes.

Section F. Tagmentation (60 minutes)

NOTE: Before using Tagmentation Buffer, you must add Protease Inhibitor Cocktail and Digitonin. For every 1 mL of Tagmentation Buffer to be used, add 10 µL Protease Inhibitor Cocktail and 2 µL of 5% Digitonin.

27. After a quick spin (<100 X g), place the tubes on a magnetic stand (30 seconds to 2 minutes) and remove the liquid with a pipette.
28. Add 125 µL Tagmentation Buffer while gently vortexing or pipetting to mix each tube.
29. Incubate tubes at 37°C for 60 minutes.

Section G. DNA Extraction (60 minutes)

30. To stop tagmentation and solubilize DNA fragments, add to each sample:

    4.2 µL 0.5 M EDTA

    1.25 µL 10% SDS

    1.1 µL Proteinase K (10 mg/mL)

31. Mix by vortexing at full speed briefly for approximately two seconds, and incubate for 60 minutes at 55°C to digest.

    NOTE: It is typical for the beads to form a large clump during incubation with Proteinase K and SDS due to the viscoelasticity of DNA. However, for abundant genome-wide epitopes, large-scale fragmentation of the genome will normally result in reduced clumping and release of beads into suspension, turning the liquid brownish relative to negative controls.

32. After a quick spin (<100 X g), place the tubes on a magnetic stand (30 seconds to 2 minutes) and transfer the liquid with a pipette into a new 1.5 mL microcentrifuge tube.

NOTE: If the sample is too viscous, preventing the supernatant from being transferred, add 625 µL DNA Purification Binding Buffer, pipette up and down, place again on the magnetic stand and transfer to a new tube. Proceed to step 34.

33. Add 625 µL DNA Purification Binding Buffer to each sample and mix by pipetting up and down. If the color indicator turns violet or orange, add 8 µL of 3 M Sodium Acetate.
34. For each sample, place a labeled DNA Purification Column into a collection tube.
35. Transfer each sample to its corresponding column, close the cap, and centrifuge at 17,000 X g (approximately 14,000 rpm) for 1 minute.
36. Discard the flow-through and return the collection tube to the column.

    NOTE: 100% Ethanol must be added to the DNA Purification Wash Buffer before the first use, to a final concentration of 80% (40 mL of 100% Ethanol to the DNA Purification Wash Buffer bottle).

37. Add 750 µL of DNA Purification Wash Buffer (prepared above) to the column and cap the column. Centrifuge at 17,000 X g for 1 minute.
38. Discard the flow-through and return the collection tube to the column. Centrifuge the empty tube at 17,000 X g for 2 minutes to remove any remaining DNA Purification Wash Buffer.
39. Transfer each column to a new microcentrifuge tube and add 35 µL of DNA Purification Elution Buffer to the center of the column matrix, cap the column, and incubate at room temperature for 1 minute.
40. Centrifuge at 17,000 X g for 1 minute. Discard the column. The DNA purification is complete, and the DNA can be stored at -20°C or you may proceed to the PCR Amplification steps below.

Section H. PCR Amplification

41. Set up the PCR reactions by adding the components below in the order shown. If libraries are to be multiplexed for sequencing, ensure that a unique i5 and 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 per CUT&TAG sample	Volume (50 µL)
Tagmented DNA	30 µL
i7 Indexing Primer (25 µM)	2.5 µL
i5 Indexing Primer (25 µM)	2.5 µL
dNTPs (10 mM)	1.0 µL
5X Q5 Reaction Buffer	10 µL
Molecular grade nuclease-free water	3.5 µL
Q5 Polymerase	0.5 µL
	Total Volume = 50 µL

42. Perform PCR using the following program on a thermal cycler with a heated lid:

    72°C for 5 minutes

    98°C for 30 seconds

    14 cycles of: 98°C for 10 seconds, 63°C for 10 seconds

    72°C for 1 minute

    Hold at 10 °C

43. Perform SPRI Bead clean-up following the steps below, per sample you will need 55 µL SPRI Beads (1.1 X sample volume), eluting in 20 µL DNA Purification Elution Buffer. 400 µL freshly-prepared 80% Ethanol will also be required per sample.
    a. Add 55 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 Purification Elution Buffer.
    k. Cap tubes and vortex to mix.
    l. Incubate samples for 5 minutes at room temperature.
    m. Apply magnet to sample and allow beads to pellet.
    n. Once the solution is clear, transfer each supernatant, containing elute DNA to a fresh, clean tube.
44. At this stage, libraries are ready for quantification and sequencing.

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.

NOTE: 2 Million to 10 Million sequencing reads are recommended. 10 Million reads typically yield 20,000 peaks in our quality control testing.

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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References

1. Kaya-Okur, H.S. *et al*. (2019) *Nature comm*. 10:1930 (1).

Troubleshooting Guide

Problem/question	Recommendation
No library visible	This is not uncommon with CUT&Tag libraries, especially for transcription factors. Libraries that are barely visible still have been shown to sequence well. qPCR analysis for library abundance (like KAPA Library Quantification) can help measure how much library is there. If your positive control works, these are still worth sequencing. If your positive control does not work, you may have lost cells during the protocol. If this may be the case, count cells bound to beads at the end of Section B to be sure cells are not lost.