CUT&Tag-IT™ Assay Kit – Tissue Manual

Catalog No. 53170 (16 rxns)

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

(version A2)

Revision	Date	Description of Change
A2	January, 2024	Tagmentation Buffer storage temp changed from -20ºC to 4°C or -20ºC
A3	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 interest[1].

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 or frozen unfixed cells or tissue 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 – Tissue is optimized for 10 mg tissue per reaction. This Kit provides optimized reagents and protocol to produce 16 unique sequencing-ready Illumina®-compatible libraries. This kit has been tested with and is recommended for heart, brain, spleen, kidney, and liver tissue.

CUT&Tag-IT™ Assay Kit – Tissue Advantages

- Compatible with 2 mg to 30 mg tissue per reaction
- Works with heart, brain, spleen, kidney, and liver tissue
- Low background signal enables lower sequencing depth
- No artifacts caused by formaldehyde crosslinking

product	format	catalog no.
CUT&Tag-IT™ Assay Kit – Tissue	16 reactions	53170

Kit Components and Storage

The kit contains sufficient reagents for 16 CUT&Tag Assay 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.

CUT&Tag Assay Kit – Tissue Reagents

Reagents	Quantity	Storage
5% Digitonin	610 µL	-20°C
CUT&Tag-IT™ Lysis Buffer – Tissue	16 mL	RT
40 µm Strainer	16 Strainers	RT
Concanavalin A Beads	320 µL	4°C
CUT&Tag-IT™ Assembled pA-Tn5 Transposomes	16 µL	-20°C
Tagmentation Buffer	2 x 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
Guinea Pig Anti-Rabbit Antibody	16 µL	-20°C
Rabbit Anti-Mouse Antibody	16 µL	-20°C
Protease Inhibitor Cocktail	3 x 500 µL	-20°C
0.5M EDTA	290 µL	RT
10% SDS	490 µL	RT
10 µg/µL Proteinase K	490 µL	-20°C
DNA Purification Columns	16 columns	RT
DNA Purification Binding Buffer	50 mL	RT
DNA Purification Wash Buffer	10 mL	RT
DNA Purification Elution Buffer	5 mL	RT
3M Sodium Acetate	500 µL	RT
10 mM DNTPs	40 µL	-20°C
5X Q5 Buffer	2 vials of 140 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

- Mouse or rabbit primary antibody to histone target of interest
- Dounce Homogenizer (1 mL, Active Motif Catalog Number 40401 or 15 mL 40415, or similar)
- Petri dish to prepare tissue sample in (30 mm to 60 mm diameter)
- Forceps to handle tissue
- Razor blade to prepare tissue
- Scale to weigh tissue sample
- Laboratory wipes such as Kimwipes
- Dry ice to keep tissue sample and tools chilled before processing
- Wet ice to keep tissue sample cool while preparing nuclei
- Method to count nuclei such as an automated cell counter or manual hemocytometer with Trypan Blue. We recommend automated cell counting methods such as the Countess II from Thermo Fisher Scientific for this assay.
- 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
- End-over-end rotator
- Vortexer
- Thermal cycler
- Illumina® 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) and 1 mL pipette
- Filter tips for pipettes

CUT&Tag Assay Kit – Tissue Protocol

This Kit is compatible with a range of tissue amounts, from 3 mg to 30 mg, and has been optimized for 10 mg tissue sample per reaction of heart, brain, liver, kidney or spleen tissue.

Tissue samples may be fresh or flash frozen on liquid nitrogen or dry ice. To flash freeze tissue samples for the assay at a later date, follow either the liquid nitrogen or dry ice steps below.

To Flash Feeze on Liquid Nitrogen

1. Excise the tissue from the animal and place in a microfuge tube.
2. Submerge in liquid nitrogen for 2 minutes.
3. Store at -80°C.

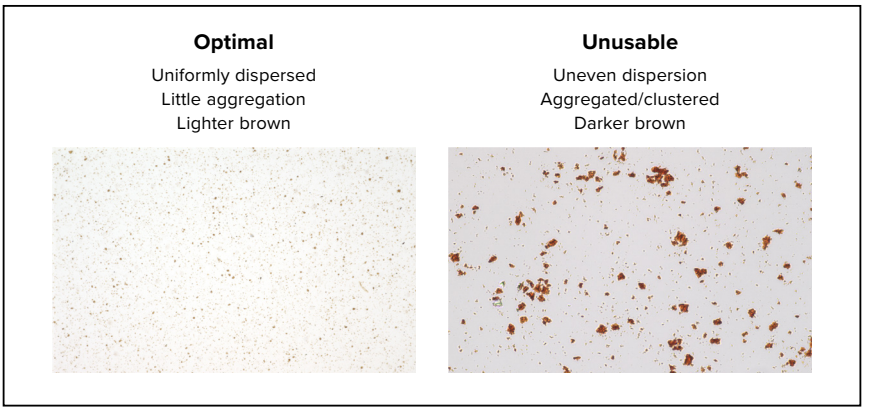
To Flash Freeze on Dry Ice

1. Excise the tissue from the animal and place in a microfuge tube.
2. Place tube on dry ice with ethanol for 15 minutes.
3. Store at -80°C.

Section A. Prepare 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.



1. 20 µL of resuspended Concanavalin A Bead slurry will be needed per tissue sample. Use 20 µL for up to 30 mg tissue. The kit has been optimized for 10 mg tissue sample reactions. The following steps are written for 1 sample. The Concanavalin A Beads can be prepared in one tube as a larger batch for multiple samples. Keep any Concanavalin A Beads not being used for sample reactions at 4°C after taking the amount needed for the samples planned in the experiment.
2. Into a 2 mL microcentrifuge 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).
3. Withdraw the liquid completely, avoiding the beads, and remove the tube from the magnetic stand. Add 1.5 mL 1 X Binding Buffer to the tube, mix by pipetting, and collect any liquid from the sides or cap with a quick pulse spin in a microcentrifuge.
4. Place tube on magnetic stand to clear (30 seconds to 2 minutes), and remove liquid by pipette, avoiding the beads. Resuspend in 20 µL 1 X Binding Buffer (20 µL per 10 mg sample) and keep the tube containing the prepared Concanavalin A Beads at room temperature until the tissue samples are ready.

Section B. Nuclei Extraction from Tissue Samples

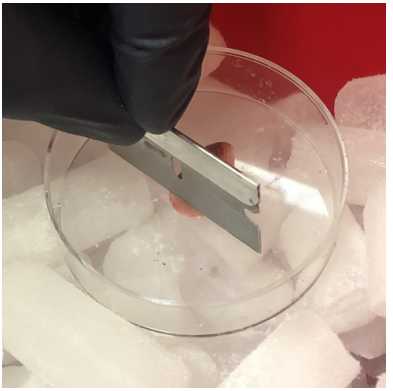
FIRST: Prepare CUT&Tag-IT™ Lysis Buffer – Tissue with fresh Protease Inhibitor Cocktail. Per sample, add 10 µL Protease Inhibitor Cocktail to 990 µL CUT&Tag-IT™ Lysis Buffer – Tissue for 1 mL total volume. Keep the freshly prepared buffer on wet ice until it is used in the steps below.

Prepare 1 X Wash Buffer with fresh Protease Inhibitor Cocktail. Per sample, add 7 µL Protease Inhibitor Cocktail to 743 µL 1 X Wash Buffer for 750 µL total volume. Keep the freshly prepared buffer on wet ice until it is used in the steps below.

5. Place Dounce Homogenizer on wet ice to chill.
6. Place petri dish on scale and tare to be ready to weigh tissue sample.
7. Place the petri dish on dry ice to be ready for the tissue sample.
8. Ethanol spray the forceps and place on dry ice to chill.
9. Ethanol spray the razor blade and carefully wipe dry with clean laboratory wipe.
10. Place tissue in petri dish and place on scale to weigh. Decide how much to cut for a 10 mg piece per assay reaction. If multiple samples of the same tissue will be assayed, the tissue can be processed in bulk. For example, if 4 reactions of the same tissue will be assayed, all 40 mg of the tissue can be processed in one petri dish.

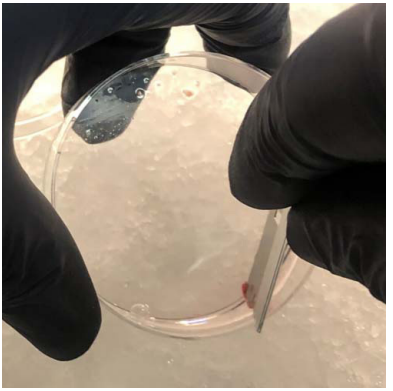
    a. To cut tissue to desired size, place petri dish with tissue back on dry ice.

b. Hold petri dish firmly and use razor to chop the tissue to smaller pieces. This is to get 10 mg per sample for the reaction. The finer chopping for tissue lysis is in step 12 below.

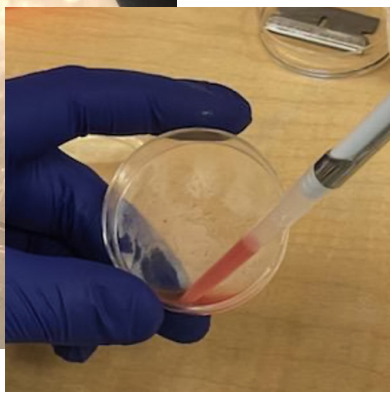


c. Place dish with tissue section on scale to check weight, and repeat adding or dividing sample and chopping until target weight is reached.

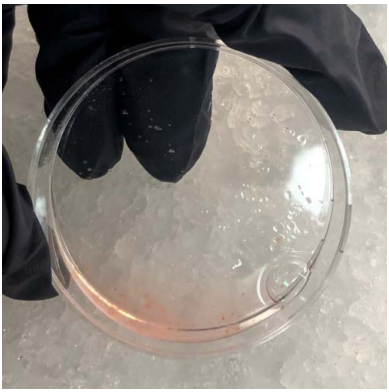
11. Place petri dish with tissue sample on wet ice. Add 1 mL of the freshly prepared CUT&Tag-IT Lysis Buffer – Tissue to the 10 mg sample. Lift one side of the petri dish so the tissue and buffer run down to the far side, while the tissue remains covered with buffer. Let sit 1 minute. If processing a larger amount of tissue for multiple reactions, add an adequate amount of CUT&Tag-IT Lysis Buffer to sufficiently cover the tissue as it is processed.



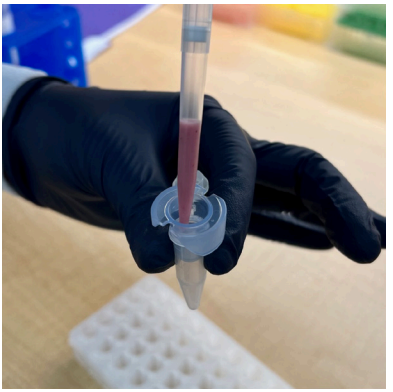
12. Partially lift the petri dish so that the tissue and the buffer run down to the corner of the dish so the tissue stays covered in the CUT&Tag-IT™ Lysis Buffer – Tissue and can be minced into smaller pieces. Using the razor blade, start slicing firmly through the tissue.



13. Lay the petri dish flat and mince all the larger pieces until no chunks of tissue can be seen. Chop until tissue pieces can no longer be chopped into smaller pieces by the razor. Mince until the pieces are small enough that they can be easily aspirated by a P1000 pipette.



14. Using the razor blade, push the finely minced tissue in the CUT&Tag-IT™ Lysis Buffer to an edge of the dish. This will make the sample easier to collect.
15. With clean and disinfected scissors, cut the narrow tip off of a 1 mL pipette tip. Use this to pipette the sample out of the petri dish and into the Dounce Homogenizer.
16. Dounce sample with tight pestle 30 times, being careful not to move the pestle above the



meniscus to prevent forming bubbles.

If the pestle is very tight and very difficult to move, first do 20 strokes with the loose pestle, then change to the tighter pestle for 10 strokes.

17. Place a 40 µm Strainer into a microcentrifuge tube on wet ice. One 40 µm Strainer and one corresponding microcentrifuge tube will be needed per 1 mL of sample. These Strainers fit 1.5 ml, 2ml microcentrifuge tubes and 15 ml conical tubes.

18. Pipette the Dounced tissue into the 40 µm Strainer on the microcentrifuge tube. Be careful to avoid large tissue pieces so the mesh does not get clogged. Gently move the 40 µm Strainer up and down inside the tube to help the sample flow through. Hold the tube with one hand and move the Strainer up and down with the other hand without fully removing the Strainer from the tube to create a void for the lysed tissue to flow into. Once the sample has moved through the Strainer, discard the Strainer.
19. Centrifuge sample tubes at 500 X g for 5 minutes at 4°C to pellet nuclei.
20. Remove supernatant from sample and discard.
21. Resuspend pelleted nuclei in 375 µL of ice cold 1 X Wash Buffer per 1.5 ml microcentrifuge tube per sample reaction being assayed. This is the nuclei suspension. If there are multiple reactions of the same sample type, pool them together to count nuclei.

    NOTE: The number of nuclei per mg will vary by tissue type. We observed an average of ~150,000 nuclei per mg. Counting accuracy is critical and we recommend an automated counting method versus a hemocytometer.

22. Take a sample of the nuclei suspension and count. Calculate nuclei density and normalize samples to a concentration of 250,000 nuclei in 375 µL 1 X Wash Buffer per sample. Add freshly prepared 1 X Wash Buffer if needed to obtain that concentration.

    NOTE: If you are normalizing to use the same amount of nuclei per reaction, we recommend CUT&Tag reactions in the range of 100,000 to 500,000 nuclei.

    NOTE: If you have excess nuclei, you may store them in a separate cryo-vial. Add DMSO to 10% of the total volume in the vial. Freeze these samples overnight in an isopropanol freezing container in a -80°C freezer. If you are planning on storing nuclei for more than 1 month, transfer these samples to a liquid nitrogen container.

23. Add 20 µL of the Concanavalin A Beads that were prepared in Section A to each sample. Incubate on an end-over-end (orbital) rotator for 10 minutes at room temperature.

Section C. Bind Primary Antibody (2 hr to Overnight)

FIRST: Before using Antibody Buffer in Step 26 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.

24. 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).
25. Place new microcentrifuge tubes (one for each sample) on ice. Remove the supernatant from the tubes in the step above via a pipette and transfer to new tubes. There will be approximately 395 µL each. These tubes contain the unbound nuclei and are not an assay reaction. Count the number of unbound nuclei via a hemocytometer.
26. Calculate the number of bound nuclei by subtracting the number of unbound nuclei from the total per reaction in step 22. This is a check to be certain nuclei are bound for the assay reactions. We typically observed a range of 0 - 4938 unbound nuclei per reactions of 250,000 - 500,000 nuclei. Resuspend bound nuclei from the tubes with the beads in step 25 in a volume of freshly prepared ice-cold Antibody Buffer such that each sample is in a 50 µL volume, with a minimum of 100,000 nuclei per 50 µL. Gently vortex. Place the tube on ice.
27. Add 1 µL (or at least 1 µg) undiluted primary antibody to each sample, and gently vortex or pipette to mix.

    IMPORTANT: Use a rabbit or 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. 39157) is a common mark that can be a positive control.

28. Incubate 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 (1.5 hours)

FIRST: Before using Dig-Wash Buffer in Step 30 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.

IMPORTANT: Choose the right secondary antibody for the reactions!

If a mouse primary antibody was used, then use the Rabbit Anti-Mouse Secondary Antibody.

If a rabbit primary antibody was used, then use the Guniea Pig Anti-Rabbit Secondary Antibody.

29. 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.
30. Dilute the 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 (making certain that the correct Secondary Antibody is used per primary antibody) while gently vortexing to dislodge the beads from the sides of the tube.
31. Place the tubes on an orbital rotator at room temperature for 60 minutes.
32. 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.
33. Add 1 mL Dig-Wash Buffer to each reaction. Gently vortex or pipette to dislodge any beads that have aggregated together.
34. Repeat Steps 32-33 twice, for a total of three washes.

Section E. Bind CUT&Tag-IT™ Assembled pA-Tn5 Transposomes (1.5 hours)

FIRST: Before using Dig-300 Buffer in Step 35 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.

35. 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.
36. 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.
37. Add 100 µL of the diluted CUT&Tag-IT™ Assembled pA-Tn5 Transposomes from Step 35 while gently pipetting to make sure it mixes together.
38. Incubate the reactions at room temperature on an orbital rotator for 60 minutes.
39. 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.
40. Add 1 mL Dig-300 Buffer. Gently vortex or pipette to dislodge the beads.
41. Repeat Step 39-40 twice for a total of three washes.

Section F. Tagmentation (60 minutes)

FIRST: Before using Tagmentation Buffer in Step 43 below, 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.

42. 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.
43. Add 125 µL Tagmentation Buffer while gently vortexing or pipetting to mix each tube.
44. Incubate tubes at 37°C for 60 minutes.

Section G. DNA Extraction (60 minutes)

45. 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)

46. 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.

47. 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.

48. 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.

49. For each sample, place a labeled DNA Purification Column into a collection tube.

50. Transfer each sample to its corresponding column, close the cap, and centrifuge at 17,000 X g (~14,000 rpm) for 1 minute.

51. Discard the flow-through and return the column to the collection tube.

    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).

52. 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.

53. 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.

54. 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.

55. 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

56. 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	2.5 µL
i5 Indexing Primer	2.5 µL
dNTPs (10 mM)	1.0 µL
5X Q5 Buffer	10 µL
Molecular grade nuclease-free water	3.5 µL
Q5 High-Fidelity DNA Polymerase	0.5 µL
	(Total Volume = 50 µL)

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

    72°C for 5 minutes

    98°C for 30 seconds

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

    72°C for 1 minute (this step follows the 12 cycles and is not part of the cycling above)

    Hold at 10 °C

58. 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 with lids open 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 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.

59. 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.

References

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

Troubleshooting Guide - See More FAQs on the Kit Web Page

Problem/question	Recommendation
What controls are recommended for CUT&Tag?	A good technical positive control for the reagents and workflow is the antibody H3K27me3 (Cat. No. 39157). For a negative control, we recommend using the secondary antibody without the primary antibody. This will show background from the secondary antibody and pA-Tn5 with your samples.
Do I need to include an IgG control?	The purpose of including an IgG control in CUT&Tag experiments is to determine if the pA-Tn5 is specific to genomic regions where the antibody is located/enriched. This negative control is not used in analysis and is different from the INPUT control used in ChIP-Seq. Active Motif R&D found that adding an IgG control does not add any additional value as the pA-Tn5 was shown to be specific. However, if you wish to add an IgG control this is fine.
Are there any QC steps recommended?	After library generation, successful library prep quality should be assessed by evaluating the library on a TapeStation or Bioanalyzer. An ideal library would have most of the fragments below 500 bp. We recommend determining the library concentration by using a KAPA Library Quantification Kit.
Does CUT&Tag require an input control like ChIP-Seq?	No. CUT&Tag does not require the use of an input control.
What antibodies have been validated using the CUT&Tag-IT Assay Kit?	For a full list of CUT&Tag-IT™ Assay Kit validated Active Motif antibodies visit: https://www.activemotif.com/catalog/1319/cut-tag-validated-antibodies
Are ChIP-Seq validated antibodies going to work with CUT&Tag?	CUT&Tag and ChIP-Seq have quite different workflows. If an antibody works for ChIP-Seq, that does not necessarily mean it will work in CUT&Tag.
Is CUT&Tag compatible with both monoclonal and polyclonal antibodies?	Yes. Please be sure to use the proper species-specific secondary antibody.
Can the standard Active Motif Spike-In Normalization be used for the CUT&Tag-IT Assay Kit?	No. Our standard ChIP-Seq Spike-In Normalization method is not compatible with the CUT&Tag-IT™ Assay Kit.

Problem/question	Recommendation
Can more than 16 samples be multiplexed?	The CUT&Tag-IT™Assay Kit is supplied with 4x4 unique dual indexes for 16 unique samples. The indexed primers in the kit are identical to the Illumina Nextera primers corresponding to N701-N704 and N501-N504. If you would like to multiplex more than 16 samples our Nextera™-Compatible Multiplex Primers (96 plex) kit (Cat. No. 53155) enables multiplexing up to 96 reactions. These primers are provided at a concentration of 25 µM to be used directly in our Kits. You could also purchase and combine other Illumina Nextera primers at the same concentration (25 µM) as those in the kit.
Is the CUT&Tag-IT Assay Kit compatible with qPCR analysis before sequencing?	Yes and no. Yes, you can do qPCR, but because of the randomness of Tn5's insertion of the adapter it makes designing primers that will prime a specific gene problematic since it is likely that you will be missing one or the other primer landing site. Therefore, qPCR may contain errors as fragments that do not have the landing site will not be measured.
Are the CUT&Tag-IT Assay Kit libraries single or dual indexed?	CUT&Tag libraries are dual-indexed libraries.
Do the CUT&Tag-IT Assay Kit libraries contain molecular identifiers?	No. CUT&Tag-IT Assay Kit libraries do not contain molecular identifiers.
Should the CUT&Tag-IT Assay Kit libraries be sequenced as single-end or paired-end?	The CUT&Tag-IT™ Assay Kit libraries should be sequenced as paired end.
What read length is recommended for the CUT&Tag-IT Assay Kit?	We recommend a read length of 2x38 (PE38). This is shorter than the read length described in the Henikoff paper. However, we have not seen an impact on data quality or mapping rates. You can use a higher read length if you wish to.
How do I analyze the sequencing data from sequencing the samples generated by the CUT&Tag-IT Assay Kit?	CUT&Tag data is analyzed similarly to ChIP-Seq data. We use a BWA algorithm with peak calling performed using MACS2.
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 nuclei 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.

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

Active Motif North America

Toll free: 877.222.9543
Direct: 760.431.1263
Fax: 760.431.1351
E-mail: tech_service@activemotif.com

Active Motif Europe

UK Free Phone: 0800/169 31 47
France Free Phone: 0800/90 99 79
Germany Free Phone: 0800/181 99 10
Direct: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050
E-mail: eurotech@activemotif.com

Active Motif Japan

Direct: +81 (0)3 5225 3638
Fax: +81 (0)3 5261 8733
E-mail: japantech@activemotif.com

Active Motif China

Direct: (86)-21-20926090
Cell Phone: 18521362870
E-mail: techchina@activemotif.com

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