ACTIVE MOTIF®

Enabling Epigenetics Research

CUT&Tag-IT® Express

Catalog Nos. 53175 & 53177

(Version A1)

Contents

Overview

CUT&Tag (Cleavage Under Targets and Tagmentation) is a streamlined, next-generation chromatin profiling method that combines antibody-based target recognition with Tn5 transposase-mediated tagmentation. This innovative assay enables simultaneous site-specific chromatin cleavage and adapter insertion, eliminating the need for traditional, multi-step library preparation. Optimized for low-input samples, CUT&Tag produces high-resolution, low-background sequencing libraries that reveal protein-DNA interactions and histone modifications with precision and reproducibility. This assay kit is designed to simplify workflows, reduce processing time, and deliver robust data for your next-generation sequencing projects.

CUT&Tag-IT® Express is a new standard in CUT&Tag epigenomic profiling. CUT&Tag-IT Express is engineered to deliver unparalleled sensitivity and reproducibility. This kit streamlines the CUT&Tag assay workflow by integrating simultaneous tagmentation and adapter incorporation into a single, efficient 0.2 mL reaction, utilizing silica beads for DNA purification for a faster workflow.

CUT&Tag-IT® Express Assay Kit Advantages:

- More consistent results
- Simplified workflow
- Higher throughput sample processing
- Includes mouse and rabbit secondary antibodies

CUT&Tag-IT® Express Assay Kit is designed to work with fresh or frozen cell samples, and will work with tissue samples processed with our Tissue Prep for NGS Assays kit (Cat No. 53185).

Product	Format	Catalog No.
CUT&Tag-IT® Express	16 rxns	53175
	48 rxns	53177

Kit Components and Storage

The kit contains sufficient reagents for 16 or 48 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.

Reagents	Quantity		Storage
	16 rxns	48 rxns	
5% Digitonin	240 µL	720 µL	-20°C
Concanavalin A Beads	160 µL	480 µL	4°C
CUT&Tag-IT™ Assembled pA-Tn5 Transposomes	16 µL	48 µL	-20°C
Tagmentation Buffer	640 µL	2 mL	4°C or -20°C
1X Binding Buffer	3.36 mL	10.1 mL	4°C
1X Wash Buffer	54.4 mL	163.2 mL	4°C
Dig-Wash Buffer	54.4 mL	163.2 mL	4°C
Antibody Buffer	800 µL	2.4 mL	4°C
Dig-300 Buffer	11.2 mL	33.6 mL	4°C
Alpaca Anti-Rabbit IgG (H+L)	16 µL	48 µL	-20°C
Rabbit Anti-Mouse Antibody	16 µL	48 µL	-20°C
Protease Inhibitor Cocktail	366.4 µL	1099.2 mL	-20°C
Glycogen	12.8 µL	38.4 µL	-20°C
10 µg/µL Proteinase K	12.8 µL	38.4 µL	-20°C
Silica Beads	400 µL	1.2 mL	4°C
DNA Purification Binding Buffer*	6 mL	6 mL	RT
DNA Purification Wash Buffer**	10 mL	10 mL	RT
DNA Purification Elution Buffer	672 µL	2.02 mL	RT
3M Sodium Acetate	128 µL	384 µL	RT
Q5 DNA Polymerase Master Mix (2U/µL)	400 µL	1.2 mL	-20°C
Tn5 Release Solution	640 µL	1.92 mL	-20°C
i5 Indexed Primer 1	10 µL	10 µL	-20°C
i5 Indexed Primer 2	10 µL	10 µL	-20°C
i5 Indexed Primer 3	10 µL	10 µL	-20°C
i5 Indexed Primer 4	10 µL	10 µL	-20°C
i5 Indexed Primer 5	N/A	10 µL	-20°C
i5 Indexed Primer 6	N/A	10 µL	-20°C
i5 Indexed Primer 7	N/A	10 µL	-20°C
i5 Indexed Primer 8	N/A	10 µL	-20°C
i5 Indexed Primer 10	N/A	10 µL	-20°C
i5 Indexed Primer 11	N/A	10 µL	-20°C

Reagents	Quantity		Storage
	16 rxns	48 rxns	
i5 Indexed Primer 13	N/A	10 µL	-20°C
i5 Indexed Primer 15	N/A	10 µL	-20°C
i7 Indexed Primer 1	10 µL	N/A	-20°C
i7 Indexed Primer 2	10 µL	N/A	-20°C
i7 Indexed Primer 3	10 µL	N/A	-20°C
i7 Indexed Primer 4	10 µL	N/A	-20°C
i7 Indexed Primer 5	N/A	30 µL	-20°C
i7 Indexed Primer 6	N/A	30 µL	-20°C
i7 Indexed Primer 7	N/A	30 µL	-20°C
i7 Indexed Primer 8	N/A	30 µL	-20°C
SPRI Beads	960 µL	2.88 mL	4°C

*DNA Purification Binding Buffer must be reconstituted to a final concentration of 60% isopropyl alcohol prior to use. Add 9 mL of 100% isopropanol to the DNA Purification Binding Buffer bottle prior to use.

**DNA Purification Wash Buffer must be reconstituted to a final concentration of 80% ethanol prior to use. Add 40 mL of 100% ethanol to the DNA Purification Wash Buffer prior to use.

Day 1 Binding Cells to Concanavalin A (ConA) Beads (2 hours)

Prepare Buffers

Prepare Complete Antibody Buffer. Protease Inhibitor Cocktail must be added fresh.

For multichannel handling, we recommend using PCR strip tubes with attached lids to reduce sample cross contamination or that new cap strip lids be used for each subsequent step.

Complete Antibody Buffer			
Ingredient	**1 rxn**	**8 rxns**	**16 rxns**
Antibody Buffer	50 µL	400 µL	800 µL
Protease Inhibitor Cocktail	0.5 µL	4 µL	8 µL
5% Digitonin	0.5 µL	4 µL	8 µL

Prepare Cells (10 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.

Note: Do not use Trypsin in CUT&Tag cell preparation. Trypsin will remove cell surface proteins needed to bind to ConA beads. TrypLE is an alternative product for releasing adherent cells from culture and will not interfere with ConA binding.

Note: Prepare ConA beads for step 8 before thawing cells to minimize the time samples will experience before permeabilization.

1. Thaw cryopreserved cells in a 37°C water bath or use fresh cells. Do not use cell pellets that were not properly cryopreserved. Count cells and check viability using a vital stain or Trypan blue on a hemacytometer or automated cell counter (for example Invitrogen Countess).
2. Aliquot 100,000 to 500,000 cells per CUT&Tag-IT® Express Assay Kit reaction to a 1.5 or 2 mL microcentrifuge tube.
3. Prepare Dig-Wash Buffer with Protease Inhibitor Cocktail. 100 µL of Dig-Wash Buffer plus 1 µL Protease Inhibitor Cocktail are needed per sample.
4. Centrifuge cells for 3 minutes at 600 x *g* at 4°C and remove the supernatant.
5. Resuspend cells in 100 µL Dig-Wash (with Protease Inhibitor Cocktail).

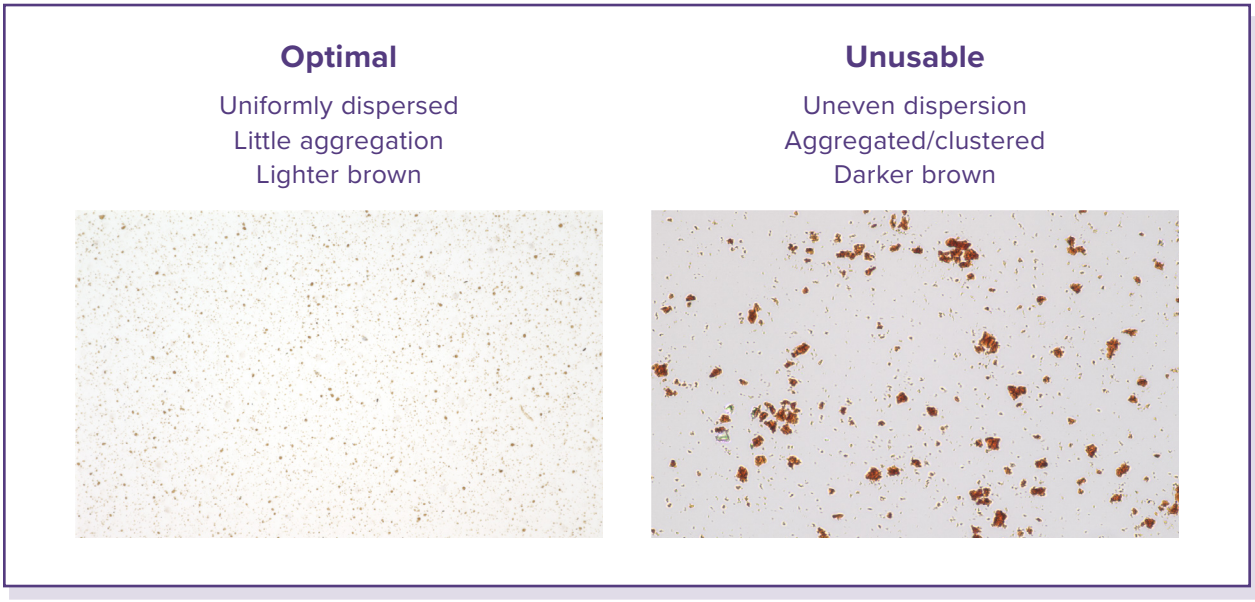
Note: An additional wash step is generally not needed.

Optional: If isolated nuclei are required resuspend each sample in 100 µL/sample cold Nuclei Isolation Buffer and store on ice for 7 minutes. Centrifuge for 3 minutes at 600 x *g* at 4°C and remove the supernatant and proceed to step 6.

Prepare Concanavalin A Beads (20 - 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: Steps 6-11 can be performed in bulk in 1.5 or 2 mL microcentrifuge tubes for large numbers of samples to save tips.

6. Resuspend the Concanavalin A Beads by vertexing. Aliquot 10 µL per sample into 0.2 mL PCR strip tubes. Resuspend beads as needed during aliquoting.
7. Place strip on plate magnet and remove supernatant.
8. Add 100 µL 1X Binding Buffer per sample and resuspend by pipette to wash.
9. Capture the beads on a magnet, and remove the supernatant.
10. Add 10 µL of 1X Binding Buffer to each of the beads tubes.
11. Add the washed cells from step 5 to the Concanavalin A Beads, resuspend, and incubate using a Nutator or orbital rotator at 20-30 RPM for 10 minutes at room temperature.

Primary Antibody Binding (Overnight)

12. Remove tubes from Nutator or orbital rotator and quickly centrifuge. Place the tubes on a magnetic stand or bar to collect beads, and discard the supernatant.
13. Resuspend the beads/cells using 50 µL of ice-cold Complete Antibody Buffer by gentle vertexing. Place tubes with samples on ice.
14. Add 1 µg of primary antibody to each sample with gentle pipetting.

Note: When testing a new antibody, titration of antibody amount may be needed to determine optimal amount of antibody.

Note: If all samples are receiving the same primary antibody the antibody can be added to a master mix of the Complete Antibody Buffer.

15. Place samples on Nutator or orbital shaker overnight at 4°C at speed of 20-30 RPM. Ensure caps are slightly elevated to avoid beads getting stuck in the lid. Liquid should remain in the bottom and on the side of the tubes while rocking.

Note: If protocol is going to be continued through subsequent steps on same day then incubation should be at room temperature for 1 hour with inversion of the strip tubes every 15 minutes to prevent bead settling.

Day 2 (4-5 Hours)

Bind Secondary Antibody

First, prepare buffers according to the table below.

Note: Thoroughly vortex the Tn5 Release Solution for 30 seconds. Prepare Complete Tn5 Release Solution by adding 12.8µL of Glycogen and 12.8µL of Proteinase K to 640µL of Tn5 Release Solution. If CUT&Tag reactions are to be performed at different times, do NOT discard the Complete Tn5 Release Solution. The solution can be stored at -20°C long term.

Complete Dig-Wash Buffer			
Ingredient	**1 rxn**	**8 rxns**	**16 rxns**
Dig-Wash Buffer	1.3 mL	10.4 mL	20.8 mL
Protease Inhibitor Cocktail	13 µL	104 µL	208 µL
5% Digitonin	13 µL	104 µL	208 µL

Note: Protease Inhibitor Cocktail must be added fresh.

16. Remove tubes from Nutator and give them a quick spin to collect samples in bottom of tubes. Then place tubes on magnetic stand or bar to pellet beads, and remove and discard the supernatant.

17. Wash the beads by adding 200 µL Complete Dig-Wash Buffer, resuspending the beads, placing the tubes back on the magnetic stand or bar, and removing and discarding the supernatant.

18. Dilute the appropriate secondary antibody (generally anti-mouse or anti-rabbit) 1:100 in Complete Dig-Wash Buffer and add 100 µL per sample. Pipette gently to resuspend pellets.

19. Place the tubes on a Nutator at room temperature for 15 minutes.

20. Invert strips 180 degrees to avoid bead settling and place back on Nutator at room at temperature for an additional 15 minutes.

21. Remove tubes from Nutator and give the tubes a quick spin to remove any liquid from the caps.
22. Place the tubes on a magnetic stand to pellet beads, and remove and discard the supernatant.
23. Add 200 µL of Complete Dig-Wash Buffer. Gently pipette to resuspend the beads.
24. Repeat **steps 22 - 23** once for a total of 2 washes, removing and discarding the supernatant after each wash.

Bind CUT&Tag-IT Assembled pA-Tn5 Transposomes (45 Minutes)

25. Mix CUT&Tag-IT Assembled pA-Tn5 Transposomes in Complete Dig-300 Buffer to a final concentration of 1:100 for 100 µL per sample.
26. After a quick spin, place tubes on a magnetic stand to pellet beads, and remove and discard the supernatant.
27. Add 100 µL of the CUT&Tag-IT Assembled pA-Tn5 Transposomes in Complete Dig-300 Buffer and gently pipette to resuspend the beads.
28. Place the tubes on a Nutator at 20-30 RPM at room temperature for 15 minutes.
29. Invert strips 180 degrees to avoid bead settling and place back on Nutator at room at temperature for an additional 15 minutes.
30. Remove tubes from nutator and do a quick spin to remove liquid from caps.
31. Place tubes on magnet or magnetic rack, remove and discard supernatant.
32. Add 200 µL Complete Dig-300 Buffer to each sample and gently pipette to resuspend the beads.
33. Repeat **steps 31 - 32** once for a total of 2 washes, removing and discarding the supernatant after each wash.

Tagmentation (1 Hour)

34. After a quick spin, place the tubes on a magnetic stand to pellet beads, and remove and discard the supernatant.
35. Add 40 µL of Complete Tagmentation Buffer. Gently pipette to resuspend the beads.
36. Incubate at 37°C for 1 hour in a water bath or incubator.

DNA Extraction (1 Hour)

37. Place samples on magnetic tray and remove supernatant.
38. Thoroughly vortex Tn5 Release Solution to uniform homogeneity and add 40 µL to each sample.
39. Mix by vortexing at full speed for 5 seconds and quick spin.

Note: It is typical for the beads to form a large clump during this incubation.

40. Incubate the reaction for 1 hour at 55°C in thermal cycler with heated lid set to 65°C. For samples fixed with formaldehyde or DSG use alternate decrosslinking incubation (1 hour 55°C, 4 hours 65°C, 4°C hold with heated lid set to 75°C).

41. Add 40 µL of water to each sample and mix gently by quick vortex to dilute Tn5 Release Solution and place back in 55C incubator for 5 minutes. (This step eases dispersal of lysed cell material.)

42. Remove from incubator and mix with pipettor until bead/cell clump is thoroughly homogenized. Set pipettor to 65 µL to avoid pulling up air.

43. Place samples on magnetic tray to separate magnetic beads from supernatant. (This can take a few minutes to complete.)

Prepare silica beads during magnetic separation of tagmented material.

44. Vortex Silica beads to homogenize.

45. Pipette 25 µL of Silica beads to each tube in strip and place on magnetic tray.

46. Remove and discard buffer from Silica beads.

47. Carefully pipette 80 µL of Tn5 Release Solution + Water supernatant, from tagmented sample, avoiding transferring solids to strip containing Silica beads and pipette up and down to fully resuspend beads.

48. To each sample add 135 µL of DNA Binding Buffer (60% isopropanol) and pipette to mix thoroughly. Let stand for 5 minutes.

49. Place strip on magnetic tray and remove and discard supernatant.

50. To each tube add 200 µL DNA Purification Wash buffer and fully resuspend Silica beads with pipetting.

51. Place strip on a magnetic tray and remove and discard supernatant.

52. Repeat **steps 50-51** for a total of 2 washes, removing and discarding the supernatant after each wash.

53. Quick spin strip and place back on magnetic plate. Use a 20 µL pipette to remove remaining DNA purification Wash Buffer and allow to air dry for 5 minutes.

54. To each tube add 22 µL of DNA Purification Elution and resuspend beads, incubate at room temperature for 1 minute.

55. Place on magnetic plate and transfer 20 µL to new strip tubes. DNA purification is complete. The purified DNA can be stored at -20°C or you may proceed directly with the PCR amplification.

PCR Amplification (1 Hour)

56. Set up the PCR reactions by adding the components in the order shown below, If libraries are to be multiplexed for sequencing, ensure that a unique i5 - i7 index combination is used for each sample.

57. Perform PCR using the following program on a thermal cycler (with a heated lid to 105°C):

72°C for 5 minutes
98°C for 30 seconds
12 cycles of:
98°C for 10 seconds
63°C for 20 seconds
72°C for 1 minute
Hold at 10°C

Note: 12 cycles is typically sufficient for most applications, for lower cell inputs and rare targets cycle number may require optimization.

Note: Resuspend SPRI beads fully before using.

Note: For post PCR steps it is recommended to use separate pipettes and store post PCR reagents separately to avoid contamination of pre-PCR reagents with contaminated pipettes.

58. Perform a double-sided SPRI bead clean-up by adding 25 µL SPRI Bead Solution (0.5X sample volume) to each sample, pipette to mix and let stand 5 minutes.

59. Place on magnetic stand and move supernatant to new strip tube, and discard the beads.

60. To the supernatant, add 35 µL SPRI Bead Solution (0.7X sample volume, bringing total to 1.2X ratio). Let stand 5 minutes.

61. Place on magnetic stand and remove and discard supernatant. Wash beads twice with 200 µL 80% Ethanol.

62. Allow beads to dry, remove from magnetic stand when the beads turn from shiny to matte in appearance, add 22 µL DNA Purification Elution Buffer, pipette to mix, and incubate for 1 minute at room temperature.

63. Place tubes on magnetic stand to pellet beads. Collect supernatant and transfer to a new tube. Libraries are now 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 Primer Name	i7 Index Name	i7 Sequence	Sample Sheet
i7 Indexed Primer 1	N701	TCGCCTTA	TAAGGCGA
i7 Indexed Primer 2	N702	CTAGTACG	CGTACTAG
i7 Indexed Primer 3	N703	TTCTGCCT	AGGCAGAA
i7 Indexed Primer 4	N704	GCTCAGGA	TCCTGAGC
i7 Indexed Primer 5	N705	AGGAGTCC	GGACTCCT
i7 Indexed Primer 6	N706	CATGCCTA	TAGGCATG
i7 Indexed Primer 7	N707	GTAGAGAG	CTCTCTAC
i7 Indexed Primer 8	N708	CCTCTCTG	CAGAGAGG

Sample Sheet Information for NovaSeq v 1.0 Reagent Kits, MiSeq, and HiSeq 2000/2500)

i5 Primer Name	i5 Index Name	i5 Sequence	Sample Sheet
i5 Indexed Primer 1	N501	TAGATCGC	TAGATCGC
i5 Indexed Primer 2	N502	CTCTCTAT	CTCTCTAT
i5 Indexed Primer 3	N503	TATCCTCT	TATCCTCT
i5 Indexed Primer 4	N504	AGAGTAGA	AGAGTAGA
i5 Indexed Primer 5	N505	GTAAGGAG	GTAAGGAG
i5 Indexed Primer 6	N506	ACTGCATA	ACTGCATA
i5 Indexed Primer 7	N507	AAGGAGTA	AAGGAGTA
i5 Indexed Primer 8	N508	CTAAGCCT	CTAAGCCT
i5 Indexed Primer 10	N510	CGTCTAAT	CGTCTAAT
i5 Indexed Primer 11	N511	TCTCTCCG	TCTCTCCG
i5 Indexed Primer 13	N513	TCGACTAG	TCGACTAG
i5 Indexed Primer 15	N515	TTCTAGCT	TTCTAGCT

Sample Sheet Information for NovaSeq v 1.5 Reagent Kits, iSeq, MiniSeq, NextSeq, and HiSeq 3000/4000)

i5 Primer Name	i5 Index Name	i5 Sequence	Sample Sheet
i5 Indexed Primer 1	N501	TAGATCGC	GCGATCTA
i5 Indexed Primer 2	N502	CTCTCTAT	ATAGAGAG
i5 Indexed Primer 3	N503	TATCCTCT	AGAGGATA
i5 Indexed Primer 4	N504	AGAGTAGA	TCTACTCT
i5 Indexed Primer 5	N505	GTAAGGAG	CTCCTTAC
i5 Indexed Primer 6	N506	ACTGCATA	TATGCAGT
i5 Indexed Primer 7	N507	AAGGAGTA	TACTCCTT
i5 Indexed Primer 8	N508	CTAAGCCT	AGGCTTAG
i5 Indexed Primer 10	N510	CGTCTAAT	ATTAGACG
i5 Indexed Primer 11	N511	TCTCTCCG	CGGAGAGA
i5 Indexed Primer 13	N513	TCGACTAG	CTAGTCGA
i5 Indexed Primer 15	N515	TTCTAGCT	AGCTAGAA

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.

North America	Toll free:	877 222 9543
	Direct:	760 431 1263
	Fax:	760 431 1351
	Email:	tech_service@activemotif.com
Europe	Direct:	+32 (0)2 653 0001
	Fax:	+32 (0)2 653 0050
	Email:	eurotech@activemotif.com
Japan	Direct:	+81 (0)3 5225 3638
	Fax:	+81 (0)3 5261 8733
	Email:	japantech@activemotif.com
China	Direct:	(86)-21-20926090
	Cell Phone:	18521362870
	Email:	techchina@activemotif.com