EpiCypher®
Bringing Epigenetics to Life

CUTANA™
ChIC / CUT&RUN Kit Version 6
User Manual Version 6.0

EpiCypher®

CUTANA™

ChIC / CUT&RUN Kit

Kit Version 6
Catalog No. 14-1048-48rxn: 48 Reactions
Catalog No. 14-1048-24rxn: 24 Reactions

**Upon receipt, store indicated components
at 4°C, -20°C and room temperature (RT)**

Stable for 12 months upon date of receipt.
See p. 7-8 for storage instructions.

EpiCypher, Inc. PO Box 14453, Durham, NC 27709
Ph: 1-855-374-2461   F: 1-855-420-6111
www.epicypher.com   Email: info@epicypher.com
Technical Support: **support.epicypher.com**   Email: techsupport@epicypher.com

24 Hour Emergency Phone Number:
US & Canada: 1-800-535-5053 | International: 1-352-323-3500

Table of Contents

See EpiCypher's Tech Support Center at support.epicypher.com for additional FAQs and troubleshooting guidance.

Background and Product Description

Cleavage Under Targets & Release Using Nuclease (CUT&RUN) is a revolutionary genomic mapping strategy developed by the group of Dr. Steven Henikoff[1]. It builds on Chromatin ImmunoCleavage (ChIC) from Dr. Ulrich Laemmli[2], wherein a fusion of Protein A to Micrococcal Nuclease (pA-MNase) is recruited to selectively cleave antibody-bound chromatin *in situ*[3].

In CUT&RUN, cells (or nuclei) are immobilized to a solid support, permeabilized, and treated with a target-specific antibody (Figure 1). pAG-MNase releases target chromatin into solution, where it is easily separated from bead-bound cells. CUT&RUN-enriched DNA is purified and prepared for next-generation sequencing to provide genome-wide profiles of histone post-translational modifications (PTMs) and chromatin proteins (e.g. transcription factors).

The CUTANA™ CUT&RUN Kit (Figure 1) includes additional controls to ensure high-quality results. The SNAP-CUTANA™ K-MetStat Panel of spike-in nucleosomes is added to positive and negative control reactions at the beginning of CUT&RUN to support assay optimization and workflow validation. After pAG-MNase digestion, *E. coli* DNA is added to allow normalization of CUT&RUN sequencing data. Visit support.epicypher.com for additional information about these key controls.
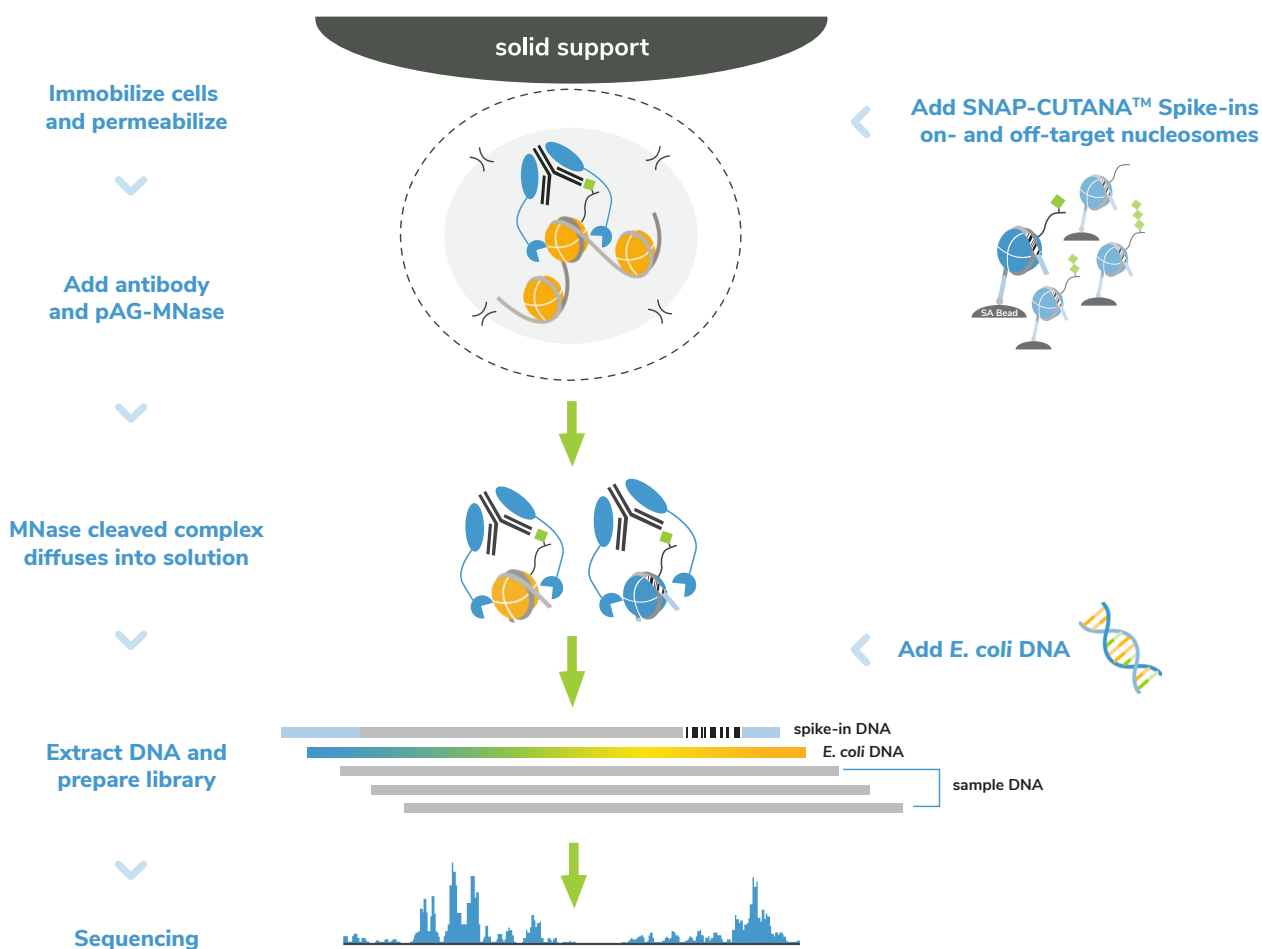
FIGURE 1

Overview of the CUTANA™ CUT&RUN protocol.

Background and Product Description

The CUTANA™ ChIC/CUT&RUN Kit contains materials for 48 reactions (EpiCypher 14-1048-48rxn) or 24 reactions (EpiCypher 14-1048-24rxn). Features of CUTANA™ CUT&RUN include:

- **A high-throughput workflow.** The protocol is designed for 8-strip tubes and multi-channel pipetting, highlighting the increased throughput made possible by CUT&RUN.

- **Low sequencing depths.** Only 3-8 million unique sequencing reads per reaction are needed to generate high-resolution chromatin profiles (Figure 2).

- **Diverse sample compatibility.** Validated for cells and nuclei, including cryopreserved and cross-linked samples (Figure 3). All sample prep protocols are available at support.epicypher.com.

- **Low cell number requirements.** Although it is recommended to use 500,000 cells per reaction, comparable data can be generated down to 5,000 cells (Figure 4).

- **Comprehensive quality control metrics.** The kit includes multiple control antibodies and spike-ins, as well as detailed success metrics, to guide you along every step of the CUT&RUN workflow. Visit support.epicypher.com for detailed explanations of controls and guidance on assay metrics.

- **Outstanding customer support.** Support.epicypher.com provides detailed information about CUT&RUN assay optimization, sample prep, recommended controls, sequencing analysis, and troubleshooting.
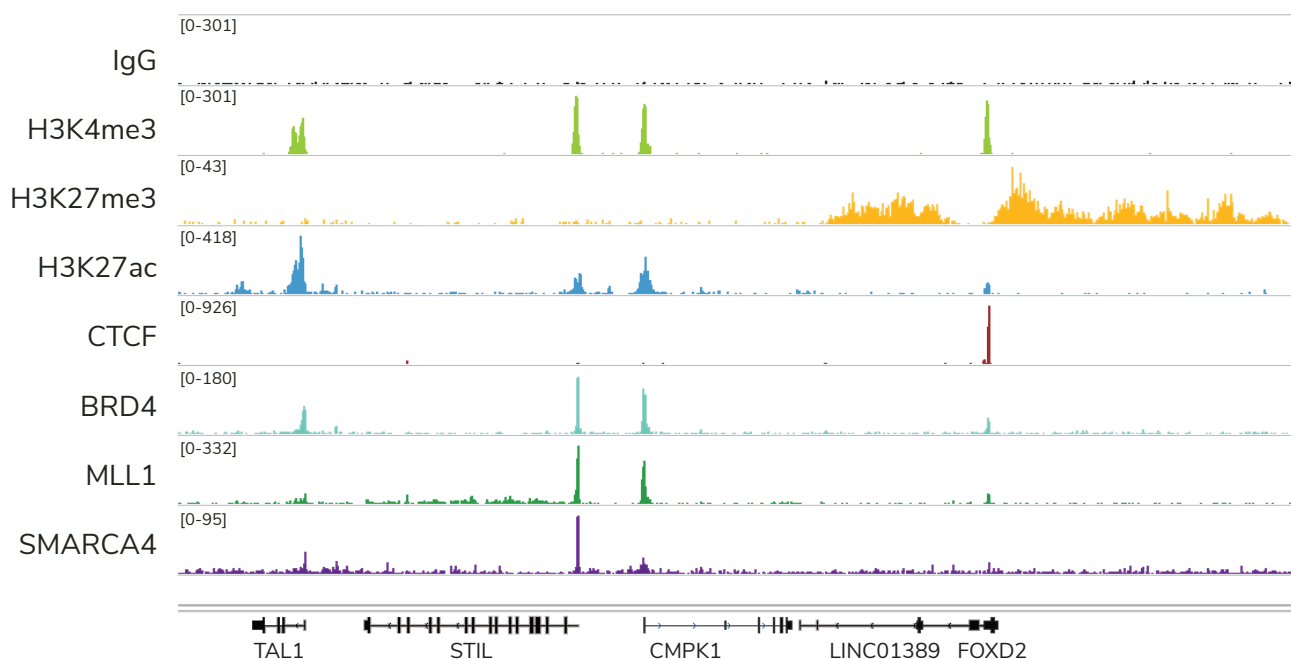
FIGURE 2

Representative genome browser tracks show CUTANA CUT&RUN results using 500,000 K562 cells. Clear peaks with expected distribution profiles are observed using 3-8 million unique sequencing reads per reaction for a variety of epigenetic targets, including histone PTMs (H3K4me3, H3K27me3, H3K27ac), transcription factors (CTCF), epigenetic reader proteins (BRD4), writer enzymes (MLL1), and chromatin remodelers (SMARCA4). Rabbit IgG antibody is shown as a negative control.

Background and Product Description

The CUTANA™ CUT&RUN Kit includes multiple controls, which should be used in **each** experiment to validate sample quality and ensure assay success. See support.epicypher.com for details on kit controls, including spike-in analysis, expected results, and their application for troubleshooting.

- **H3K4me3 Positive Control Antibody.** This control provides a representative example of a low-abundance target, with sharp peaks, that is localized at active transcription start sites (TSS).

- **H3K27me3 Positive Control Antibody.** This control offers an example of a high-abundance target with broad peaks that is enriched at transcriptionally inactive genes.

- **IgG Negative Control Antibody**. Used to assess nonspecific background signal.

- **SNAP-CUTANA™ K-MetStat Panel** (16 DNA-barcoded nucleosomes carrying widely studied lysine methylation PTMs). The K-MetStat Panel is spiked into each control reaction to directly monitor experimental success and aid troubleshooting.

- *E. coli* **Spike-in DNA**. Added to all reactions after pAG-MNase cleavage to control for library prep and enable sequencing normalization.

FIGURE 3

Heatmaps of CUTANA CUT&RUN data show H3K4me3 enrichment (red) and background (blue) flanking annotated transcription start sites (TSS, +/- 2 kb). Gene rows are aligned across conditions, showing that genome-wide enrichment is preserved across sample types.
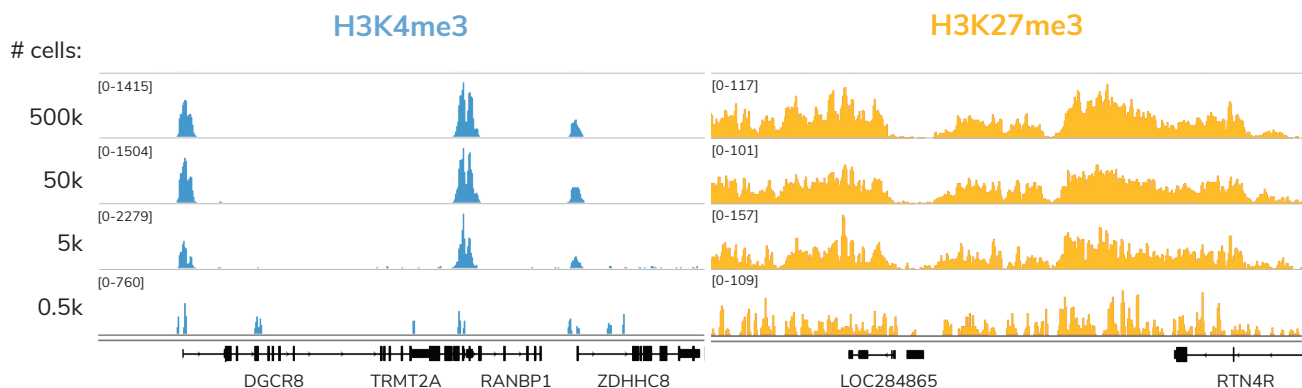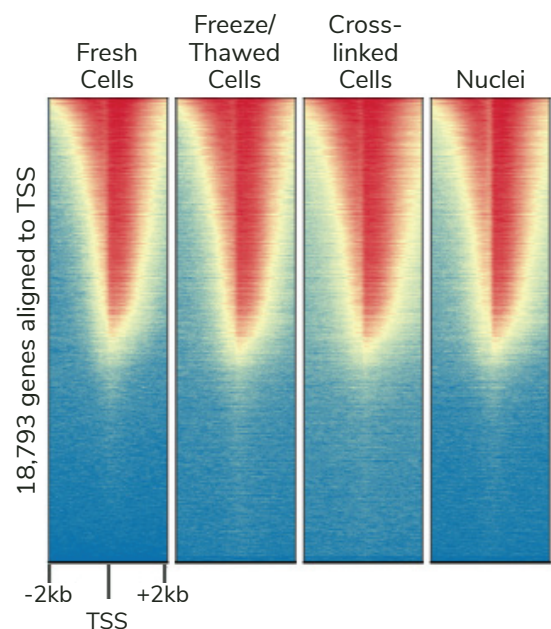
FIGURE 4

Representative genome browser tracks for H3K4me3 (low-abundance control target) and H3K27me3 (high-abundance control target) CUT&RUN experiments using decreasing amounts of K562 cells. At 5,000 cells, data is largely indistinguishable from assays using 500,000 cells.

Included in the Kit

Kit components are stable for 12 months upon date of receipt. Store as outlined below.

Store at room temperature (RT) upon receipt:

Item	14-1048-48rxn	14-1048-24rxn	Notes before use
8-strip Tubes	10-0009k	10-0009-01	Enables use of multi-channel pipettors.
0.5 M EDTA	21-1006k	21-1006-01	Use to prepare Antibody Buffer.
100 mM Calcium Chloride	21-1007k	21-1007-01	Activates pAG-MNase to cleave DNA.
DNA Purification Beads	21-1407k	21-1407-01	**DO NOT FREEZE.** Slightly viscous; thoroughly mix and pipette carefully to ensure correct volume.
0.1X TE Buffer	21-1025k	21-1025-01	Use to elute CUT&RUN DNA.

Store at 4°C upon receipt:

Item	14-1048-48rxn	14-1048-24rxn	Notes before use
ConA Beads	21-1401k	21-1401-01	**DO NOT FREEZE.** Use to immobilize cells or nuclei for CUT&RUN.
Bead Activation Buffer	21-1001k	21-1001-01	Use to prepare ConA beads.
Pre-Wash Buffer	21-1002k	21-1002-01	Use to prepare CUT&RUN Buffers.
Stop Buffer	21-1003k	21-1003-01	Use to terminate pAG-MNase activity.
Rabbit IgG Negative Control Antibody	13-0042k	13-0042-02	**SMALL VOLUME: quick spin before use.** 0.5 mg/mL stock - add 1 µL per reaction. Sufficient for 8 reactions.
H3K4me3 Positive Control Antibody	13-0060k	13-0060-02	**SMALL VOLUME: quick spin before use.** 0.5 mg/mL stock - add 1 µL per reaction. Sufficient for 8 reactions.
H3K27me3 Positive Control Antibody	13-0055k	13-0055-02	**SMALL VOLUME: quick spin before use.** 0.5 mg/mL stock - add 1 µL per reaction. Sufficient for 8 reactions.
Wash Buffer Enhancer 1	21-1028k	21-1028-01	Buffer additives to reduce sample clumping and improve bead handling.
Wash Buffer Enhancer 2	15-1030k	15-1030-01	

Included in the Kit

Kit components are stable for 12 months upon date of receipt. Store as outlined below.

Store at -20°C upon receipt:

Item	14-1048-48rxn	14-1048-24rxn	Notes before use
5% Digitonin	21-1004k	21-1004-01	Thaw at RT. Use to prepare Cell Permeabilization and Antibody Buffers. Final Digitonin concentration should be optimized for each sample type. Visit support.epicypher.com for a detailed protocol.
1 M Spermidine	21-1005k	21-1005-01	Use to prepare Wash Buffer.
SNAP-CUTANA™ K-MetStat Panel	19-1002k	19-1002-02	**SMALL VOLUME: quick spin before use. Pipette to resuspend Panel — DO NOT VORTEX.** Panel of biotinylated nucleosomes coupled to streptavidin-coated magnetic beads. Pair with IgG, H3K4me3, and H3K27me3 control antibodies. Sufficient for 24 reactions. For more information, visit support.epicypher.com.
pAG-MNase	15-1016k	15-1016-01	20X concentration. Proteins A and G (pAG) bind antibodies of various isotypes and host species including total IgG for rabbit, mouse, goat, donkey, rat, guinea pig, horse, and cow.
E. coli Spike-in DNA	18-1401k (100 ng; reconstitute in 200 µL water)	18-1401-01 (50 ng; reconstitute in 100 µL water)	Lyophilized E. coli DNA. Before first use, quick spin and reconstitute in DNase-free water to 0.5 ng/µL. Add to all reactions for sequencing normalization.

Materials Required but Not Supplied

REAGENTS:

- Target-specific antibody. Visit epicypher.com/products/antibodies/ or contact techsupport@epicypher.com for recommendations. See support.epicypher.com for guidance on CUT&RUN antibody validation.

- CUTANA™ Protease Inhibitor Tablets (EpiCypher 21-1027)

- 1X PBS

- 0.4% Trypan Blue (e.g. Invitrogen T10282)

- 100% Ethanol (200 proof), any vendor

- Molecular biology grade water, any vendor

- Library prep kit (CUTANA™ CUT&RUN Library Prep Kit, EpiCypher 14-1001)

- Optional: Additional SNAP-CUTANA™ K-MetStat Panel (EpiCypher 19-1002) for experimental reactions targeting histone lysine methylation PTMs. Download the User Guide to learn more.

- Optional: CUTANA™ Nuclei Extraction Buffer (EpiCypher 21-1026), if isolating nuclei.

- Optional: CUTANA™ Multiomic CUT&RUN Controls Set (EpiCypher 14-1802) to simultaneously detect DNA methylation associated with chromatin protein targets of interest in a single workflow. Requires alternate library prep approach - download the User Manual to learn more.

EQUIPMENT:

- 1.5, 15, and 50 mL tubes

- Low-retention filter pipette tips

- Magnetic separation rack for 1.5 mL tubes and 8-strip tubes (EpiCypher 10-0012 and 10-0008)

- 8-channel multi-pipettor (e.g. VWR 76169-250) and multi-channel reagent reservoirs (e.g. Thermo Fisher Scientific 14-387-072)

- Vortex (e.g. Vortex-Genie® 2, Scientific Industries SI-0236)

- Benchtop centrifuge/mini-centrifuge with an 8-strip tube adapter (e.g. from Fisher Scientific)

- Tube nutator for incubation steps (e.g. VWR 82007-202)

  * A **nutator** rocks tubes gently, without end-over-end rotation. Rotating tubes traps liquid in tube caps, resulting in ConA bead dry out and sample loss - do **NOT** rotate tubes.

- Thermocycler with heated lid (e.g. from BioRad, Applied Biosystems, Eppendorf)

- Qubit™ 4 Fluorometer (or previous version) and 1X dsDNA HS Kit (Invitrogen Q33230)

- Capillary electrophoresis machine and required reagents, e.g. Agilent TapeStation® with D1000 ScreenTape (5067-5582) and D1000 reagents (5067-5583) or Agilent Bioanalyzer® with High Sensitivity DNA Kit (5067-4626)

Here, we review the main steps of the CUTANA™ CUT&RUN assay:

Step 1: Isolate cells and immobilize to concanavalin A (ConA) beads

Cells are harvested, washed, and bound to activated ConA beads. High-quality sample prep is essential to CUT&RUN workflows. Note that modifications are suggested for certain sample types (frozen cells, nuclei, adherent cells, tissues), which can be found at support.epicypher.com. The protocol includes detailed steps to confirm the quality of samples at initial cell harvest, after wash steps, and following ConA bead binding. Avoid bead dry out and clumping during the assay, which results in sample loss and reduced CUT&RUN yields.

Step 2: Permeabilize cells and incubate with target-specific antibody

The bead-cell mixture is resuspended in cold Antibody Buffer and a target-specific antibody is added for overnight incubation at 4˚C. Permeabilization is required for antibody binding *in situ*, and is achieved by addition of Digitonin to assay buffers. Digitonin concentrations must be optimized for each cell type, following the protocol available at support.epicypher.com.

Selection of an antibody with high target specificity and efficiency is crucial to CUT&RUN success. The CUTANA™ CUT&RUN Kit is validated for robust profiling of histone PTMs, transcription factors, remodeling enzymes, chromatin writers and readers, and epitope-tagged proteins.

Step 3: Perform targeted chromatin digestion and release

The next day, reactions are washed several times to remove unbound antibody. pAG-MNase is added and binds antibody-labeled chromatin via the immunoglobulin binding properties of pAG. Addition of calcium activates MNase, which cleaves DNA proximal to where the antibody is bound.

Clipped fragments diffuse into the supernatant, while bulk chromatin remains in bead-immobilized cells. The Stop Buffer containing EDTA is added to chelate free calcium and halt MNase activity.

Step 4: Purify CUT&RUN-enriched DNA

Bead-bound cells are removed using a magnet. CUT&RUN-enriched DNA is purified from supernatant using a DNA purification bead strategy optimized for mononucleosome-sized fragments. The use of beads allows the protocol to stay in 8-strip tubes, improving throughput and reliability. Purified DNA is quantified using the Qubit Fluorometer.

Step 5: CUT&RUN library prep and next-generation sequencing

CUT&RUN DNA is prepared for Illumina® sequencing using the CUTANA CUT&RUN Library Prep Kit (or equivalent). Libraries are sequenced on an Illumina sequencing platform. Only 3-8 million uniquely aligned reads (5-10 million total reads) are needed to generate robust CUT&RUN profiles.

SAMPLE INPUTS FOR CUT&RUN

- Freshly isolated, unfixed (i.e. native) cells are the preferred input for CUT&RUN.

- 500,000 cells per reaction is recommended. Harvest 10% excess cells to account for sample loss and allow for quality control checks.

- See support.epicypher.com if using nuclei, adherent cells, tissues, or cryopreserved samples.

    - Cross-linking may be useful for labile targets, such as histone lysine acetylation PTMs or transiently interacting chromatin proteins.

    - Isolation of nuclei is recommended for tissue samples, immune cells, and cells prone to lysis and/or clumping. Use the CUTANA™ Nuclei Extraction Buffer (EpiCypher 21-1026).

IMPORTANT NOTE ON SAMPLE QUALITY

- High quality sample prep is essential for CUT&RUN success. Check cell count, viability, and morphology at initial cell harvest and before ConA bead binding. Count washed cells to ensure cells are not being lost during the centrifugation (spin longer if needed).

- Confirm cell binding to ConA beads — a critical part of the CUT&RUN workflow.

- On **Day 2**, it is important that ConA beads are resuspended during pAG-MNase incubation and digestion. Excessive clumping leads to sample loss and poor yields.

COMPATIBLE TARGETS AND CONSIDERATIONS FOR DNA PURIFICATION

- CUT&RUN is robust for most targets we have tested, including histone PTMs, transcription factors, chromatin reader proteins, modifying enzymes, and ATP-dependent remodeling enzymes.

ANTIBODY SELECTION

- Antibodies that work well in ChIP-seq are **NOT** guaranteed success in CUT&RUN.

- Visit epicypher.com/products/antibodies/ to shop our entire list of CUT&RUN-validated antibodies or email techsupport@epicypher.com for recommendations.

- For new targets, test antibodies from multiple vendors, as detailed in Figure 5. Select the antibody that gives the best balance of expected target enrichment, low background, and high yields.

- Lysine methylation PTM antibodies can be directly validated in CUT&RUN using the SNAP-CUTANA™ K-MetStat Panel (EpiCypher 19-1002).

- We also offer SNAP-CUTANA Spike-in Controls and validated CUT&RUN antibodies for HA and DYKDDDDK epitope-tagged proteins (see epicypher.com/antibodies).

ASSAY OPTIMIZATION OVERVIEW

- CUT&RUN optimization is reviewed in Figure 5. See support.epicypher.com for guidance.

- CUT&RUN success depends on many factors, including cell type, cell number, target abundance, and antibody quality.

- Using low cell numbers may result in lower signal and increased background, including for control antibodies. In addition, an antibody that performs reliably at 500,000 cells may fail at lower numbers of cells.

- 5 ng CUT&RUN-enriched DNA is recommended for library prep. For low CUT&RUN yields, use as much DNA as possible for library prep.

Establish CUT&RUN workflows for each cell type

- Optimize Digitonin permeabilization conditions for cell type
- Perform CUT&RUN using 500,000 native cells, control antibodies, and K-MetStat Panel
- Confirm CUT&RUN workflow success using quality control checks described in protocol

↓

Pause here if initial CUT&RUN experiments with control antibodies do not work.
Contact techsupport@epicypher.com for guidance.

↓

Identify target-specific antibody for CUT&RUN

- Test 3-5 antibodies to target, derived from unique clones and/or distinct epitopes
- Use 500,000 native cells per reaction
- Include reactions with control antibodies and K-MetStat Panel to confirm assay success
- Select antibody with high specificity, efficiency, and expected target enrichment

↓

Validate CUT&RUN for experimental conditions

- Confirm robust signal for target under experimental conditions (e.g. drug treatments)
- Use 500,000 cells per reaction
- Include reactions with control antibodies and K-MetStat Panel to confirm assay success
- This step can be combined with antibody testing, if desired

↓

Optimize CUT&RUN for low inputs

- Titrate the number of cells and examine signal for target
- Include reactions with control antibodies and K-MetStat Panel to confirm assay success
- Our CUT&RUN protocol has been validated for select targets down to 5,000 K562 cells

FIGURE 5

Development and optimization guidelines for successful CUT&RUN workflows.

EXPERIMENTAL CONTROLS

- The kit contains multiple quality control checks (Figure 6) and defined controls (below) to ensure reliable assays. See support.epicypher.com for guidance on controls and success metrics.
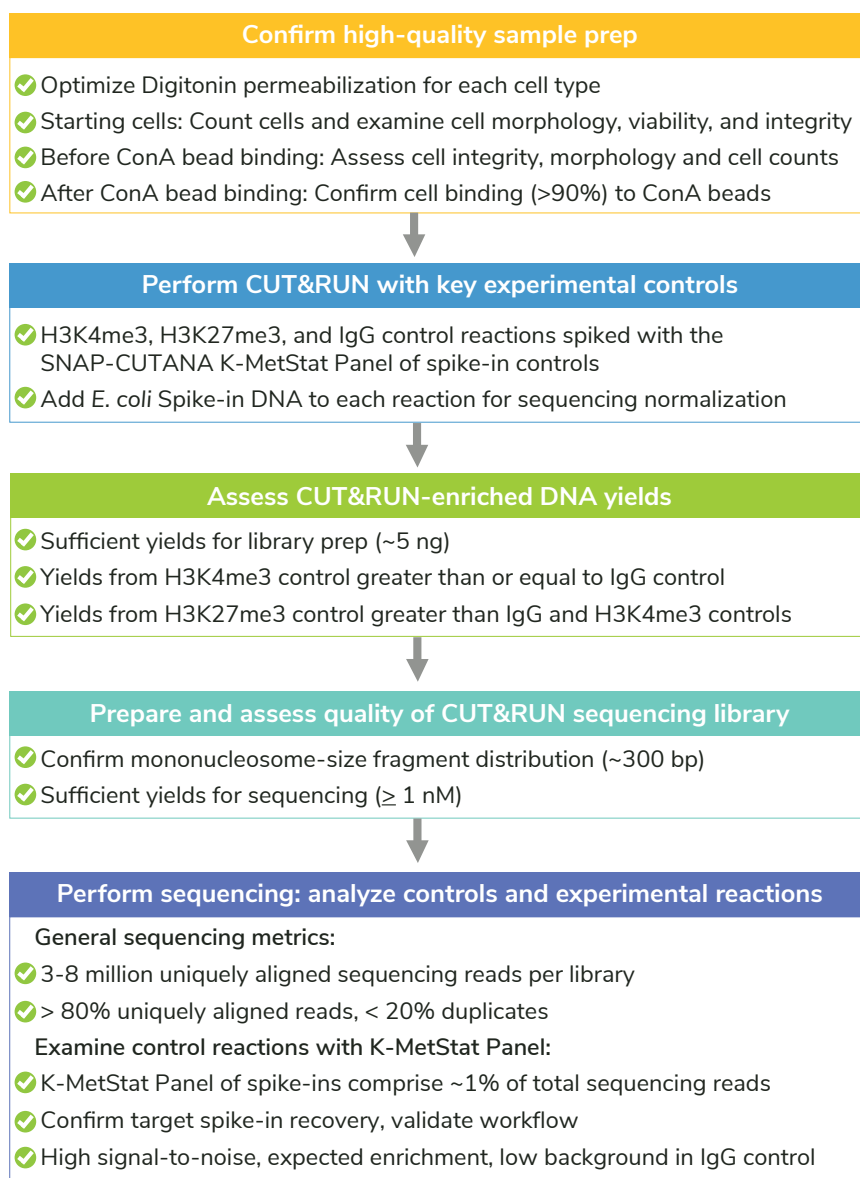
  - **H3K4me3 and H3K27me3 Positive Control Antibodies** represent targets of varying abundance (low vs. high), peak structures (sharp vs. broad), and genomic enrichment (active vs. repressed chromatin), thus providing deep insights into data quality.

  - **IgG Negative Control Antibody** is used to assess nonspecific background signal and is particularly useful for peak calling.

  - SNAP-CUTANA™ K-MetStat Panel, 16 DNA-barcoded nucleosomes with defined lysine methylation PTMs. The K-MetStat Panel is spiked into each control reaction to validate the CUT&RUN workflow. See this article on the Tech Support Center for information. **Control reactions spiked with SNAP-CUTANA™ K-MetStat Panel should be used in every experiment to determine assay success and aid troubleshooting.**

Confirm high-quality sample prep
- ✔ Optimize Digitonin permeabilization for each cell type
- ✔ Starting cells: Count cells and examine cell morphology, viability, and integrity
- ✔ Before ConA bead binding: Assess cell integrity, morphology and cell counts
- ✔ After ConA bead binding: Confirm cell binding (>90%) to ConA beads

Perform CUT&RUN with key experimental controls
- ✔ H3K4me3, H3K27me3, and IgG control reactions spiked with the SNAP-CUTANA K-MetStat Panel of spike-in controls
- ✔ Add *E. coli* Spike-in DNA to each reaction for sequencing normalization

Assess CUT&RUN-enriched DNA yields
- ✔ Sufficient yields for library prep (~5 ng)
- ✔ Yields from H3K4me3 control greater than or equal to IgG control
- ✔ Yields from H3K27me3 control greater than IgG and H3K4me3 controls

Prepare and assess quality of CUT&RUN sequencing library
- ✔ Confirm mononucleosome-size fragment distribution (~300 bp)
- ✔ Sufficient yields for sequencing ($\geq$ 1 nM)

Perform sequencing: analyze controls and experimental reactions
General sequencing metrics:
- ✔ 3-8 million uniquely aligned sequencing reads per library
- ✔ > 80% uniquely aligned reads, < 20% duplicates

Examine control reactions with K-MetStat Panel:
- ✔ K-MetStat Panel of spike-ins comprise ~1% of total sequencing reads
- ✔ Confirm target spike-in recovery, validate workflow
- ✔ High signal-to-noise, expected enrichment, low background in IgG control

FIGURE 6

The CUTANA™ CUT&RUN Kit contains multiple quality control metrics and checks to ensure successful chromatin profiling.

SECTION I: CUT&RUN BUFFER PREP (~30 MIN)

IMPORTANT NOTES ON BUFFER PREP

* Buffers (Figure 7) are prepared **FRESH** on Day 1 of every CUT&RUN experiment.

* Volumes in Table 1 are per CUT&RUN reaction and include 20% excess to account for pipetting errors. You do **NOT** need to add additional volume.

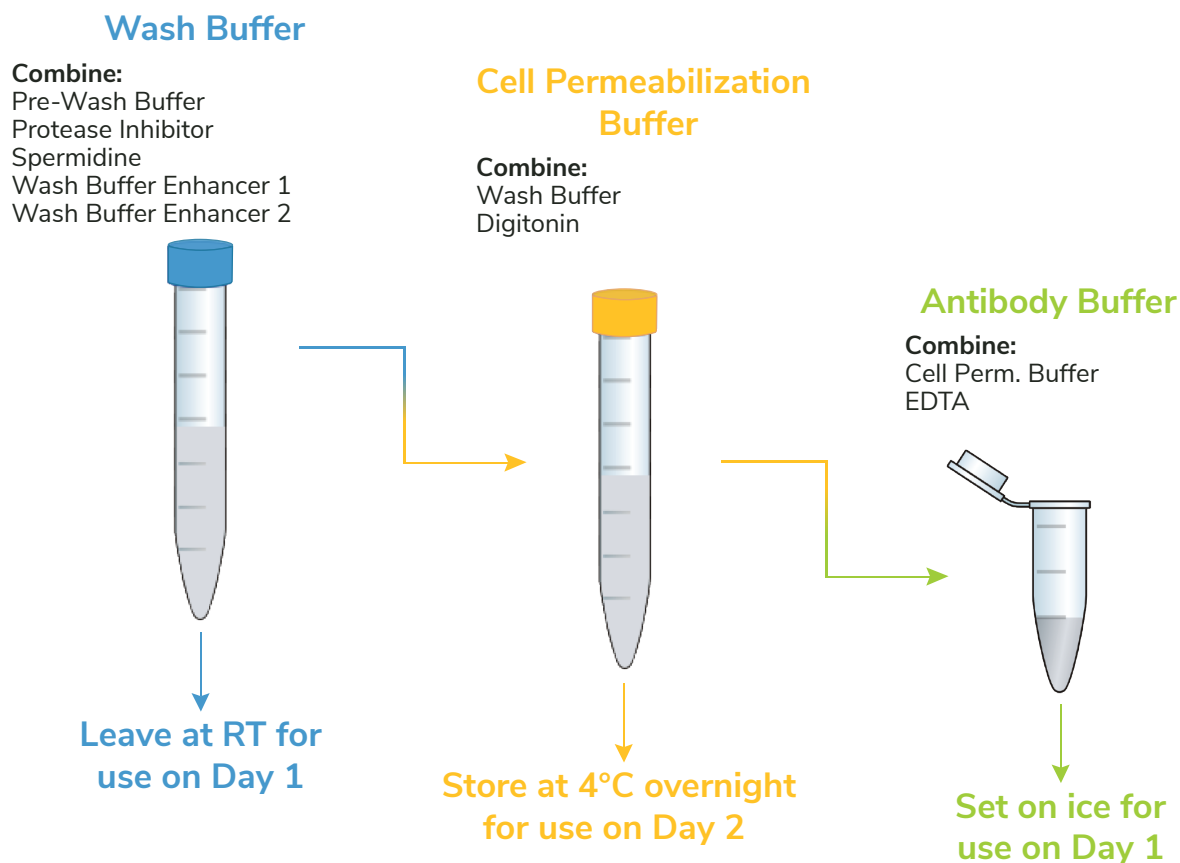* Add Wash Buffer Enhancers 1 and 2 to reduce sample clumping and improve bead handling.

Wash Buffer

Combine:
Pre-Wash Buffer
Protease Inhibitor
Spermidine
Wash Buffer Enhancer 1
Wash Buffer Enhancer 2

Cell Permeabilization Buffer

Combine:
Wash Buffer
Digitonin

Antibody Buffer

Combine:
Cell Perm. Buffer
EDTA

Leave at RT for use on Day 1

Store at 4°C overnight for use on Day 2

Set on ice for use on Day 1

FIGURE 7

Schematic of buffers prepared on Day 1 of the CUT&RUN protocol. **RT**, room temperature.

1. Gather kit reagents stored at 4°C and -20°C needed for Day 1: **ConA Beads, Bead Activation Buffer, Pre-Wash Buffer, Digitonin, Spermidine, Wash Buffer Enhancer 1, Wash Buffer Enhancer 2, K-MetStat Panel; H3K4me3, H3K27me3, and IgG Control Antibodies**. Place on ice to thaw or equilibrate.

2. Reconstitute protease inhibitor tablet (EpiCypher 21-1027) as instructed in product data sheet or on product page to prepare a **25X Protease Inhibitor** stock. After buffer prep, the remaining 25X stock can be stored for 12 weeks at -20°C.

Experimental Protocol: Day 1

3. Prepare **Wash Buffer** by combining Pre-Wash Buffer; 25X Protease Inhibitor; 1M Spermidine; 1,000X Wash Buffer Enhancer 1; and 2,500X Wash Buffer Enhancer 2 as outlined in Table 1. Store final buffer at room temperature (RT).

4. To a new tube labeled **Cell Permeabilization Buffer**, add Wash Buffer as outlined in Table 1. Add 5% Digitonin as optimized for your cell type*****. Place **Cell Permeabilization Buffer** on ice.

    * Calculations in Table 1 are for 0.01% Digitonin, which is optimal for most cell types tested at EpiCypher (K562, MCF7, A549, NIH3T3, LNCaP), and is recommended for reactions using nuclei. We recommend optimizing Digitonin concentrations for efficient permeabilization. See support.epicypher.com for instructions.

5. In a new 1.5 mL tube labeled **Antibody Buffer**, combine Cell Permeabilization Buffer and 0.5 M EDTA as described in Table 1. Place final buffer on ice.

6. Store remaining **Cell Permeabilization Buffer** at 4°C for use on Day 2.

Buffer Sample Scaling Calculations:

COMPONENT	[FINAL]	1rxn	8rxn	16rxn
Wash Buffer - store at room temperature (RT) for use on Day 1				
Pre-Wash Buffer	-	1.8 mL	14.4 mL	28.8 mL
25X Protease Inhibitor	1X	72 µL	576 µL	1.15 mL
1 M Spermidine	0.5 mM	0.9 µL	7.2 µL	14.4 µL
1,000X Wash Buffer Enhancer 1	1X	1.8 µL	14.4 µL	28.8 µL
2,500X Wash Buffer Enhancer 2	1X	0.72 µL	5.76 µL	11.5 µL
Cell Permeabilization Buffer - store at 4°C for use on Day 2				
Wash Buffer	-	1.4 mL	11.2 mL	22.4 mL
5% Digitonin	0.01%	2.8 µL	22.4 µL	44.8 µL
Antibody Buffer - store on ice for use on Day 1				
Cell Permeabilization Buffer	-	100 µL	800 µL	1.6 mL
0.5 M EDTA	2 mM	0.4 µL	3.2 µL	6.4 µL

TABLE 1

Combine reagents as instructed in the table to prepare CUT&RUN Buffers. Calculations for 8 and 16 reactions are provided. All buffers include 20% extra volume to account for pipetting error - no additional overage is needed.

SECTION II: CONA BEAD ACTIVATION (~30 MIN)

TIPS FOR WORKING WITH CONA BEADS

* Do **NOT** use ConA beads that have been frozen and/or appear black, granular, or clumpy.

* Do **NOT** let ConA beads dry out. Avoid disturbing beads with pipette while on magnet.

* Activated ConA beads should be kept on ice and used within four hours of activation.

7. Gently resuspend **ConA Beads** and transfer 11 µL per reaction to a 1.5 mL tube.

8. Place tube on a magnet, allow slurry to clear. Pipette to remove supernatant.

9. Remove tube from magnet. Immediately add 100 µL per reaction cold **Bead Activation Buffer** and pipette to resuspend. Return tube to magnet, allow slurry to clear, and pipette to remove supernatant.

10. Repeat the previous step one time.

11. Resuspend beads in 11 µL per reaction cold **Bead Activation Buffer**. Place on ice.

SECTION III: BINDING CELLS TO ACTIVATED BEADS (~30 MIN)

SAMPLE PREP NOTES

* **High-quality sample prep is essential to CUT&RUN.** This protocol includes steps to check the quality of starting cells, washed cells, and cells bound to ConA beads. Perform these steps in every experiment to ensure robust CUT&RUN sequencing data.

* The CUTANA CUT&RUN protocol is designed for native suspension cells. If using nuclei, adherent cells, cross-linked samples, frozen nuclei/cells, or other sample types, visit support.epicypher.com for protocol modifications.

12. Spin cells at 600 x g for 3 min at room temperature (RT). Pipette to remove supernatant, flick tube to loosen pellet, and resuspend cells in 1 mL 1X PBS.

13. Transfer 10 µL cells to a fresh tube.

14. Add 10 µL 0.4% Trypan Blue to the 10 µL aliquot of cells. Pipette 10 times to mix.

15. Transfer 10 µL of the cell-Trypan Blue mixture to a cell counting slide. Obtain cell counts, determine viability (>80% is ideal), and confirm expected cellular morphology using a brightfield/phase microscope or cell counter. See Figure 8A (p. 18).

16. Harvest 500,000 cells per reaction in a fresh tube. To account for sample loss and allow quality checks, it is recommended to collect 10% excess cells.

17. Spin cells 600 x g, 3 min, RT. Pipette to remove supernatant. If cells are being lost during spins, increase spin time.

18. Resuspend cells in 100 µL per reaction RT **Wash Buffer** by gentle yet thorough pipetting. Spin at 600 x g, 3 min, RT. Pipette to remove supernatant.

19. Repeat the previous step one time.

20. Resuspend cells in 105 µL per reaction RT **Wash Buffer**.

21. To determine total cell counts and evaluate cell integrity, transfer 10 µL cells to a new tube. Perform Trypan Blue staining as in Steps 14-15. Cells should be unclumped and free of cellular debris, with clear borders (see Figure 8B). Total cell counts should be at ~500,000 cells per reaction.

22. To washed cells, add 10 µL per reaction **activated ConA beads**. Gently vortex to mix and quick spin in a mini-centrifuge to collect slurry (beads should not settle).

23. Incubate bead-cell slurry for 10 min at RT. Cells will adsorb to beads.

24. After the 10 min incubation, place tube on magnet and allow slurry to clear.

25. If bead binding was successful, the supernatant should not contain cells. To confirm, transfer 10 µL supernatant to a new 1.5 mL tube. Perform Trypan Blue staining as in Steps 14-15. See Figure 8C for expected results.

26. Pipette to remove and discard remaining supernatant.

27. Remove tube from magnet and immediately add 55 µL per reaction cold **Antibody Buffer**. Pipette to resuspend. Note that Antibody Buffer contains Digitonin, which permeabilizes cells.

28. To confirm cell permeabilization and binding to ConA beads, transfer 10 µL bead slurry to a new 1.5 mL tube. Perform Trypan Blue staining as in Steps 14-15. Cells should be blue and surrounded by ConA beads, as in Figure 8D.

29. Aliquot 50 µL bead slurry per reaction to **8-strip Tubes**.

Starting cells, 96% viable — A

Washed cells, 89% viable — B

Supernatant with few cells — C

Permeabilized cells coupled to beads — D
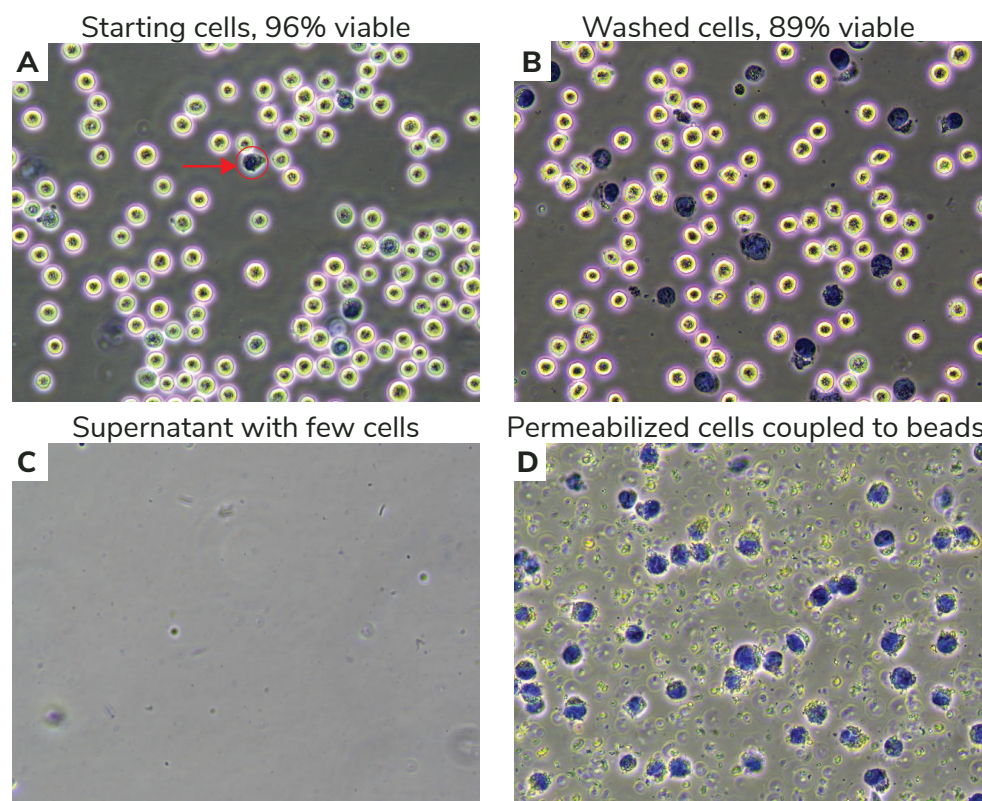
Sample	Success Metrics	Troubleshooting Tips
Starting cells Figure 8A	Cells show expected morphology, are unclumped, and >80% viable. Acceptable viability depends on cell type and experimental conditions. A dead cell is circled in red.	Evaluate cell culture conditions; use fresh media and troubleshoot contamination issues. Increase spin time if losing sample. See support.epicypher.com for specific recommendations for your sample type (tissues, adherent cells, etc.)
Washed cells Figure 8B	Total cell counts are ~500,000 cells per reaction. Cells show clear borders, are unclumped, and free of cell debris. Minimal lysis is observed.	Cell viability may be reduced following resuspension in Wash Buffer. Instead, focus on total cell counts. Confirm cells are intact, with expected morphology and minimal lysis.
Cells coupled to ConA beads Figure 8C,D	Supernatant **(C)** contains few cells. The slurry **(D)** contains permeabilized (Trypan Blue positive) cells bound to beads (brown specks).	Ensure that ConA beads were never frozen, cells were not clumped, beads did not become clumped or dried out, and all buffers were correctly prepared.

FIGURE 8

Validation of K562 cell sample prep using Trypan Blue staining.

SECTION IV: ANTIBODY BINDING (~30 MIN + OVERNIGHT)

ANTIBODY BINDING NOTES

* Add K-MetStat Panel to control reactions **BEFORE** adding the primary antibody.
See support.epicypher.com for additional guidance on using these important controls.

* Do **NOT** rotate or invert tubes. Rotation causes ConA beads to stick to tube sides and dry out, reducing yields. Use a nutator for incubations and elevate tube caps as suggested.

30. Quick spin the **K-MetStat Panel**. Pipette to resuspend - do **NOT** vortex stock.

31. Add the **K-MetStat Panel** to reactions designated for H3K4me3, H3K27me3, and IgG Control Antibodies. Add 2 µL if using 500,000 cells per reaction.

    For lower cell numbers, prepare a dilution of the K-MetStat Panel in **Antibody Buffer** on the day of the experiment, as outlined below. Add 2 µL diluted K-MetStat Panel to each reaction.

Number of cells	Panel dilution	Volume per reaction
500,000	Use stock	2 µL
250,000	1:2	2 µL
100,000	1:5	2 µL
50,000 or fewer	1:10	2 µL

32. Gently vortex tubes and quick spin. Add 0.5 µg primary antibody (or manufacturer's recommendation) to each experimental reaction.

    For control reactions, add 1 µL respectively of **H3K4me3, H3K27me3, or IgG Control Antibody**.

    For antibodies stored in viscous glycerol solutions, ensure accurate pipetting: aspirate slowly, check tip for accuracy, and pipette up and down to clear the solution from tip.

33. Gently vortex to mix and quick spin. Incubate overnight at 4°C on a nutator, gently rocking with tube caps elevated (Figure 9). Do **NOT** invert or rotate tubes end-over-end.
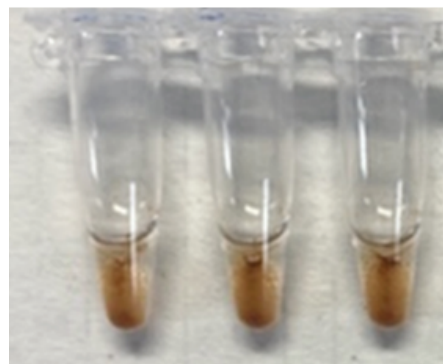

FIGURE 9

8-strip tubes should be placed on a nutator at a 45 degree angle with caps elevated.

SECTION V: BINDING OF PAG-MNASE (~40 MIN)

IMPORTANT NOTES ON CONA BEAD MIXING

* **Resuspension of ConA beads is essential for CUT&RUN success.** Beads often become clumpy after overnight incubation. Mix as instructed in each step to keep beads in solution, ensuring even distribution of pAG-MNase.

34. Gather reagents at 4°C and -20°C for Day 2: **Cell Permeabilization Buffer, *E. coli* Spike-in DNA, pAG-MNase, Stop Buffer**. Place on ice to thaw or equilibrate.

35. If using a multi-channel pipettor (recommended), place a multi-channel reagent reservoir on ice. Fill with cold **Cell Permeabilization Buffer**. Throughout the protocol, remove and replace buffers one tube strip at a time to avoid bead dry out.

    Retrieve a compatible 8-strip tube magnetic rack. If using the EpiCypher 8-strip tube magnet, use the high-volume side unless otherwise noted.

36. Remove tubes from 4°C incubation and quick spin to collect liquid. Note that beads may settle overnight (Figure 10), but this will not impact results.

37. Place tubes on magnet and allow slurry to clear. Pipette to remove supernatant.

38. Keeping tubes on magnet, add 200 µL cold **Cell Permeabilization Buffer** to each reaction. Pipette to remove supernatant. Repeat one time, keeping tubes on magnet.

39. Remove tubes from magnet and immediately add 50 µL cold **Cell Permeabilization Buffer** to each reaction. Gently vortex and/or pipette to mix. If pipetting, avoid losing beads in tips and expel all material back into tubes. Always quick spin after mixing to avoid bead loss.

FIGURE 10

Settling of ConA beads after overnight incubation at 4°C.

40. Add 2.5 µL **pAG-MNase** to each reaction. Vortex ~5 sec to thoroughly mix beads and evenly distribute enzyme.

41. Quick spin tubes and incubate reactions for 10 min at RT.

42. Quick spin tubes, place on magnet, and allow slurry to clear. Pipette to remove supernatant.

43. Keeping tubes on magnet, add 200 µL cold **Cell Permeabilization Buffer** directly onto beads. Pipette to remove supernatant. Repeat one time, keeping tubes on magnet.

44. Remove tubes from magnet. Immediately add 50 µL cold **Cell Permeabilization Buffer** to each reaction. Resuspend by pipetting (avoid bead loss, expel all material back into tubes) and/or vortexing, followed by a quick spin.

SECTION VI: TARGETED CHROMATIN DIGESTION AND RELEASE (~3 HRS)

GUIDELINES FOR *E. COLI* SPIKE-IN DNA

* Reconstitute lyophilized *E. coli* **Spike-in DNA** in DNase-free water prior to first use. Quick spin tube before opening to collect DNA. For 48-reaction kit, add 200 µL water; for 24-reaction kit, add 100 µL water. Thoroughly vortex tube on all sides to resuspend. Store at -20˚C.

* This protocol is optimized for 500,000 cells per reaction. If using fewer than 500,000 cells per reaction, dilute *E. coli* **Spike-in DNA** as outlined in this Tech Support Center article.

45. Place tubes on ice. Add 1 µL **100 mM Calcium Chloride** to each reaction. Gently vortex ~5 sec to evenly resuspend beads and ensure efficient digestion.

46. Quick spin tubes. Incubate on a nutator, capped ends elevated, 2 hours at 4°C.

47. Retrieve *E. coli* **Spike-in DNA**. Reconstitute DNA prior to first use (Guidelines, above) or thaw previously resuspended DNA on ice and quick spin before use.

48. Prepare a **Stop Master Mix** in a 1.5 mL tube: per reaction, combine 33 µL **Stop Buffer** and 1 µL *E. coli* **Spike-in DNA** (0.5 ng). Gently vortex to mix.

49. At the end of the 2 hour incubation, quick spin tubes to collect liquid. Add 33 µL **Stop Master Mix** to each reaction and gently vortex ~5 sec to mix.

50. Quick spin tubes and place in a thermocycler set to 37˚C. Incubate for 10 min.

51. Quick spin tubes to collect liquid and place on a magnet until slurry clears.

52. Transfer supernatants containing CUT&RUN DNA to new **8-strip Tubes**.

SECTION VII: DNA PURIFICATION (~30 MIN)

IMPORTANT NOTES ON DNA PURIFICATION

* CUTANA™ DNA Purification Beads are supplied as a drop-in replacement to SPRI-based DNA cleanup beads included in previous kit versions.

* Our new Version 6 protocol now uses a higher bead-to-sample ratio (1.8X vs. prior 1.4X) to capture a wider range of fragment sizes, including mononucleosome fragments (147 bp) and smaller fragments (<120 bp) generated by some transcription factors. This ratio change is independent of the beads change. See this Tech Support Center article for details.

53. Prepare 85% Ethanol (EtOH) fresh using a 100% EtOH and molecular biology grade water. Make 500 µL per reaction: 425 µL 100% EtOH + 75 µL water. Note that these calculations include extra volume to account for pipetting error.

54. Vortex **DNA Purification Beads** to fully resuspend.

55. Slowly add 151 µL **DNA Purification Beads** (1.8X volume) to each reaction. Ensure pipette tip is free of extra droplets before dispensing beads to reactions.

56. <u>Mix well</u> by pipetting and/or vortexing to an even resuspension (critical for bead binding). Quick spin tubes and incubate 5 min at RT.

57. Place tubes on a magnet for 2-5 min at RT, until solution clears. Pipette to remove supernatant without disturbing beads.

58. Keeping tubes on the magnet, add 180 µL **85% EtOH** directly onto beads. Pipette to remove supernatant.

59. Repeat the previous step one time.

60. Remove tubes from magnet. Quick spin to collect liquid, with caps facing in, to avoid dislodging beads. Return to magnet and pipette to remove residual EtOH.

61. Remove tubes from magnet. Air-dry, caps open, 2-3 min at RT. Beads should be damp matte brown (Figure 11). If beads are crackly and/or light brown, they are too dry.

Too wet | Ideal | Too dry

FIGURE 11

Elute DNA before beads dry out.

62. Add 17 µL **0.1X TE Buffer** to each reaction to elute DNA. Pipette and/or vortex to fully resuspend beads and quick spin. Incubate 2 min at RT.

63. For EpiCypher magnet, flip to low-volume side.

64. Quick spin tubes and place on magnet for 2 min at RT.

65. Transfer 15 µL CUT&RUN-enriched DNA to new **8-strip Tubes**.

66. Quantify 1 µL DNA using the Qubit fluorometer and 1X dsDNA HS Assay Kit.

    Safe pause point. Store DNA at -20˚C or continue to library prep.

EXPECTED RESULTS AND FAQS: CUT&RUN YIELDS

What yields can I expect from CUT&RUN?
There is no typical DNA yield for CUT&RUN, as yields vary by cell type, number of cells, target abundance, and antibody quality. Aim for ≥5 ng DNA to enable robust library prep. If yields are low, use as much as possible for library prep. We strongly recommend proceeding to library prep regardless of yield, as even very low raw yields can produce adequate libraries for sequencing. See support.epicypher.com for guidance.

Yields from positive and negative controls can also be used to gauge assay success. H3K27me3 should have higher yields compared to IgG. H3K4me3, a low abundance target, should have yields similar to or just slightly greater than IgG.

Can I use TapeStation or Bioanalyzer to examine CUT&RUN DNA yields?
Do **NOT** assess fragment size distribution of raw CUT&RUN DNA before library prep. Yields are too low for detection on Bioanalyzer/TapeStation and **will not provide useful information at this step**. Wait until after library prep.

CUT&RUN LIBRARY PREP - FAQS

What is the single BEST method to confirm CUT&RUN success?

Fragment distribution analysis of **purified libraries** on TapeStation or Bioanalyzer. Libraries should show enrichment of mononucleosome-sized DNA fragments (~300 bp, including CUT&RUN DNA + sequencing adapters). See Figure 12.

How should I prepare CUT&RUN sequencing libraries?

Prepare Illumina sequencing libraries using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001) and ~5 ng CUT&RUN DNA.

If yields are <5 ng, use total amount of recovered DNA for library prep. Our scientists frequently observe low yields for low-abundance targets, including H3K4me3 and IgG control reactions, and use the full volume of DNA for library prep. See support.epicypher.com for additional guidance regarding low yields.

If using other library prep kits, follow EpiCypher's parameters for indexing PCR and library amplification, which are specifically optimized for CUT&RUN. For guidance, see the CUTANA™ CUT&RUN Library Prep Kit manual at epicypher.com/protocols.

SEQUENCING CUT&RUN LIBRARIES - FAQS

What is the recommended sequencing depth and read length?

Paired-end sequencing (2 x 50 bp minimum) is recommended for CUT&RUN. Libraries should be sequenced to a depth of 3-8 million uniquely aligned reads (5-10 million total reads). For IgG control samples, aim for at least 1 million uniquely aligned reads.

What are standard CUT&RUN sequencing metrics?

The majority of reads (>80%) should align uniquely to the species genome. Sequence duplication levels should be low (<20% of total sequencing reads).

How do I analyze control reactions spiked with the SNAP-CUTANA™ K-MetStat Panel?

See support.epicypher.com for guidance on SNAP-CUTANA Spike-in data analysis. The K-MetStat Panel should comprise ~1% of total reads. Controls should show expected enrichment and peaks. Experimental replicates should be highly reproducible (Figures 13-14).

How do I use *E. coli* Spike-in DNA for sequencing normalization?

See support.epicypher.com for instructions. *E. coli* DNA should comprise ~1% of total reads.

EpiCypher now supports bioinformatic analysis of CUT&RUN and CUT&Tag data with **CUTANA™ Cloud** (cloud.epicypher.com), a fast, secure, user-friendly cloud computing platform to help researchers rapidly unlock insights from their data (see Appendix). CUTANA Cloud automates genomic alignment, SNAP-CUTANA Spike-in analysis, and compilation of all necessary QC statistics to determine experimental success. See Tech Support Center at support.epicypher.com for additional FAQs and troubleshooting guidance.

EXAMPLE FRAGMENT DISTRIBUTION TRACE OF CUT&RUN SEQUENCING LIBRARIES
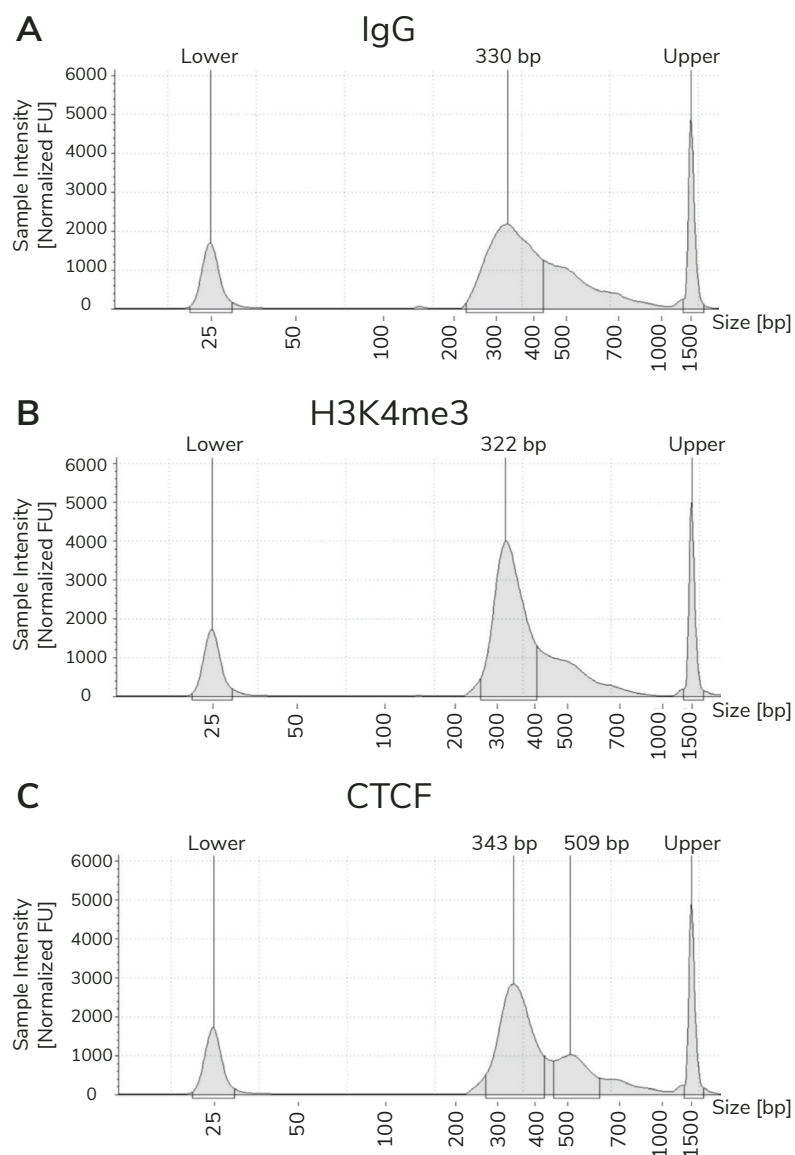
FIGURE 12

Typical TapeStation traces from CUTANA™ CUT&RUN libraries prepared using antibodies targeting IgG, H3K4me3, and CTCF (EpiCypher 13-2014). All libraries are predominantly enriched for mononucleosome-sized fragments, as indicated by the peak at ~300 bp (~170 bp nucleosomes + sequencing adapters).

See EpiCypher's Tech Support Center at support.epicypher.com for additional FAQs and troubleshooting guidance.

EXAMPLE DATA FROM POSITIVE AND NEGATIVE CONTROL REACTIONS
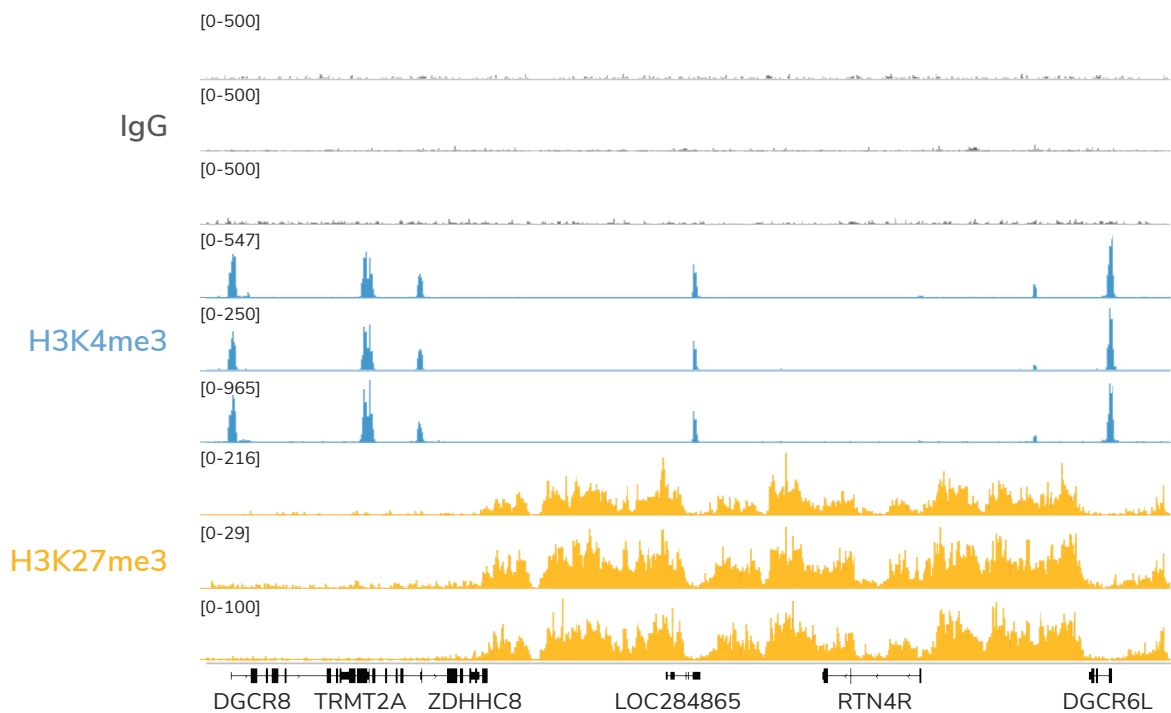
FIGURE 13

Data generated by three independent users demonstrate the reproducibility of the CUTANA CUT&RUN Kit. CUT&RUN was performed using 500,000 K562 cells and antibodies to IgG, H3K4me3, and H3K27me3. 3-6 million unique reads were generated per library. H3K4me3 tracks show sharp peaks localized to transcription start sites (TSSs), while H3K27me3 tracks show broad peaks over repressed regions. IgG shows typical low background.

FIGURE 14

Expected results from CUTANA CUT&RUN assays using 500,000 K562 cells with antibodies to IgG, H3K4me3, and H3K27me3. Data are presented as a heatmap of signal intensity aligned to the TSS of 18,793 genes (+/- 2kb). Genes are aligned across conditions and ranked by H3K4me3 intensity from top (high signal, red) to bottom (low signal, yellow). These data show that H3K4me3, a mark of active gene transcription, is enriched proximal to the TSS and is anti-correlated with H3K27me3, a mark of transcriptional repression. IgG shows low nonspecific background signal.
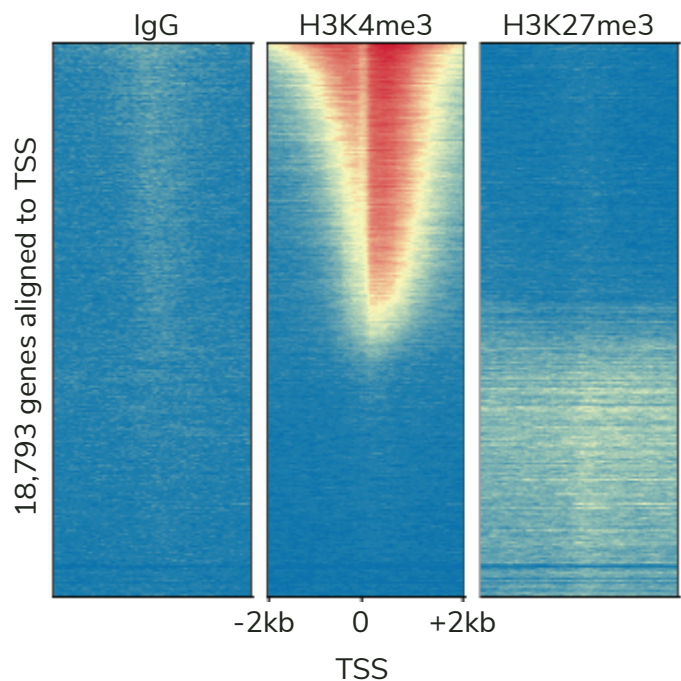
Appendix: CUT&RUN data analysis with CUTANA™ Cloud

CUTANA Cloud (cloud.epicypher.com) is a web-based platform for streamlined analysis of CUT&RUN and CUT&Tag data with EpiCypher's thoroughly tested pipelines. It takes you from raw sequencing files through alignment and peak calling without the need for command line expertise (Figure 15).

UPLOAD DATA   ➢ *Import directly from BaseSpace™*

✎ **Annotate** reactions with experimental metadata

ⓘ **OUTPUT: FASTQC report** to assess sequencing quality

ALIGN TO GENOME   ➢ *Human, Mouse, Drosophila, Yeast*

🚦 **Choose** reference genome and go
→ Select advanced parameters available

ⓘ **OUTPUT: Alignment QC report** to assess experimental success

CALL PEAKS   ➢ *MACS2 or SICER2 (narrow/broad)*

🚦 **Choose** peak caller, expected peak size, and go
→ Select advanced parameters available

ⓘ **OUTPUT: Peak calling QC report** to assess target enrichment

DERIVE INSIGHTS   ➢ *On-platform IGV browser*

🔍 **Visualize results** using genome browser

📄 **Download** all files in bulk

Ready for local tertiary analyses

Figure 15 CUTANA Cloud Analysis Pipeline.

To access the fast and streamlined bioinformatic analysis of CUT&RUN data with CUTANA Cloud, follow the steps below:

1. Create an account

   - Sign up at cloud.epicypher.com
   - Browse the gold standard reference data
   - Set up a billing account to purchase credits

2. Prepare your experiment

   - Create a new project & experiment
   - Upload FASTQ files directly from BaseSpace, AWS, or local files
   - Enter reaction metadata to set pipeline instructions (e.g., targets, spike-ins)

3. Configure and Launch Analysis

- Select desired reactions
- Select reference genome (hg19, hg38, T2T, mm10, dm6, sacCer3)
- Confirm or adjust default parameters (e.g., duplicate handling, exclusion lists)
- Select peak calling algorithms (e.g., MACS2, SICER)

4. Collect and evaluate results from QC reports (Figure 16)

- Sequencing statistics and peak calling metrics
- Analysis of SNAP-CUTANA Spike-in controls
- In browser IGV tracks
- Heatmaps from individual targets

5. Export & Explore

- Dynamic run methods ready for publication
- Download processed data files (BAM, BED, bigWig) for further visualization & tertiary analysis
- Projects remain accessible in your Cloud workspace for comparison or re-analysis

A

Short Name	Total Read Pairs	Aligned Read Pairs	Uniquely Aligned Read Pairs	Unique Alignment Rate(%)	Duplication Rate(%)	chrM Bandwidth(%)	Ecoli Read Pairs	Ecoli Alignment Rate(%)	CUTANA Spike in(%)
C&R_IgG_Rep1	3453013	2781321	2631532	76.21	4.84	1.81	411963	11.93	2.96
C&R_IgG_Rep2	3777358	3314338	3142523	83.19	4.65	1.11	273477	7.24	2.09
C&R_H3K4me3_Rep1	4717558	4367742	3947766	83.68	8.78	0.39	112410	2.38	2.53
C&R_H3K4me3_Rep2	6931540	6422725	5655071	81.58	11.14	0.39	150514	2.17	2.55
C&R_H3K27me3_Rep1	6593797	6468790	6149470	93.26	4.43	0.06	16282	0.25	0.32
C&R_H3K27me3_Rep2	4739679	4647468	4420450	93.26	4.38	0.06	10830	0.23	0.31
C&R_CTCF_Rep1	3925820	3646031	3374250	85.95	6.84	0.86	113847	2.9	N/A
C&R_CTCF_Rep2	4551115	4236786	3877841	85.21	7.86	0.87	122772	2.7	N/A

B

C

D

Figure 16 Select QC report figures exported from CUTANA Cloud using example CUT&RUN reactions with IgG, anti-H3K4me3, anti-H3K27me3, and anti-CTCF antibodies in duplication. **(A)** Sequencing stats table. **(B)** Relative barcode recovery from each applicable antibody compared to the on-target panel member. For IgG negative control, recovery is evenly spreaded across all panel members. **(C)** Peaks called against IgG control (H3K4me3 and CTCF through MACS2, and H3K27me3 through SICER2) categorized by overlapping known genomic features. **(D)** Heatmaps for read enrichment centered around Transcription Start Sites (TSS) of protein coding genes, each individually sorted by signal strengths.

References

1. Skene & Henikoff. An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites. **eLife** 6 (2017).

2. Schmid et al. ChIC and ChEC; genomic mapping of chromatin proteins. **Mol Cell** 16, 147-157 (2004).

3. Skene et al. Targeted *in situ* genome-wide profiling with high efficiency for low cell numbers. **Nat Prot** 13, 1006-1019 (2018).

Revision History

Kit Manual Version #	Date	Notes
6.0	11.2025	• Updated Catalog Numbers for CUTANA™ CUT&RUN kits. • Added **Wash Buffer Enhancers 1 & 2** to reduce sample clumping and improve bead handling. See p. 15 for buffer prep instructions. • New **CUTANA™ DNA Purification Beads** as a drop-in replacement to SPRI-based DNA purification beads. • Updated pack sizes for **Control Antibodies** and **SNAP-CUTANA™ K-MetStat Panel**. • Updated recommendation for storage temperature of **Rabbit IgG Negative Control Antibody** to 4˚C. • Updated protocol for DNA purification to use higher standard bead-to-sample ratio to improve capture of small fragements. See p. 21 & p. 22 for important notes on the change.