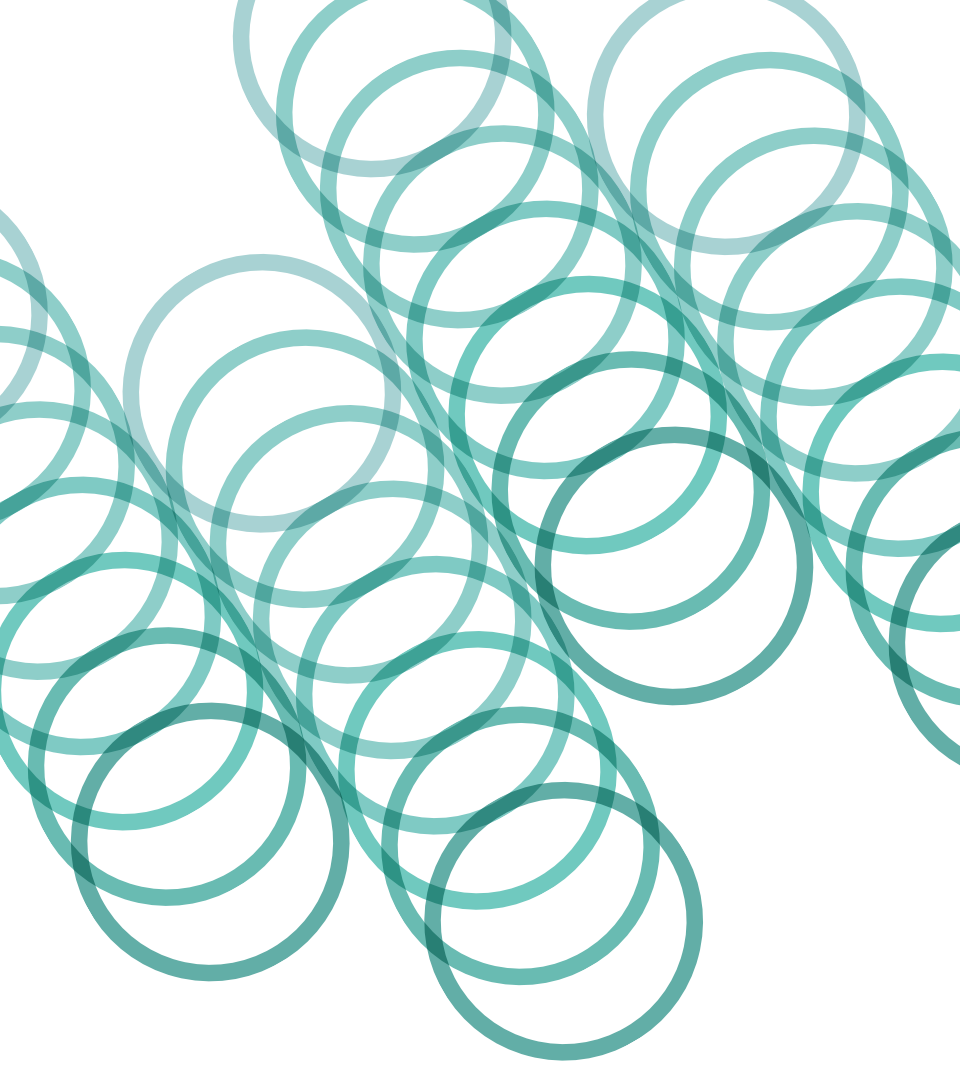

EpiCypher®
Bringing Epigenetics to Life

CUTANA™
DNA Purification Kit Version 2
User Manual Version 2.0

EpiCypher, Inc.
PO Box 14453
Durham, NC 27709

www.epicypher.com
Ph: 1-855-374-2461 F: 1-855-420-6111
Email: info@epicypher.com
Tech Support: techsupport@epicypher.com

EpiCypher®

CUTANA™

DNA Purification Kit

Kit Version 2
Catalog No. 14-0050
50 Reactions

Upon receipt, store indicated components at room temperature (RT)

Table of Contents

Isolation and sequencing of DNA associated with chromatin-regulatory networks is a powerful tool for understanding gene regulation. A novel technology commercialized by EpiCypher under the CUTANA™ platform for genomic mapping assays, Cleavage Under Targets & Release Using Nuclease (CUT&RUN) enables genomic mapping with unprecedented sensitivity[1]. CUT&RUN is an adaptation of Chromatin ImmunoCleavage (ChIC) technology which utilizes an immunotethering approach to specifically release genomic fragments of interest into solution (Figure 1)[2]. Using this approach, background is dramatically reduced compared to standard chromatin immunoprecipitation (ChIP) approaches, enabling high quality data to be produced using a fraction of the cell numbers and sequencing depth[3].

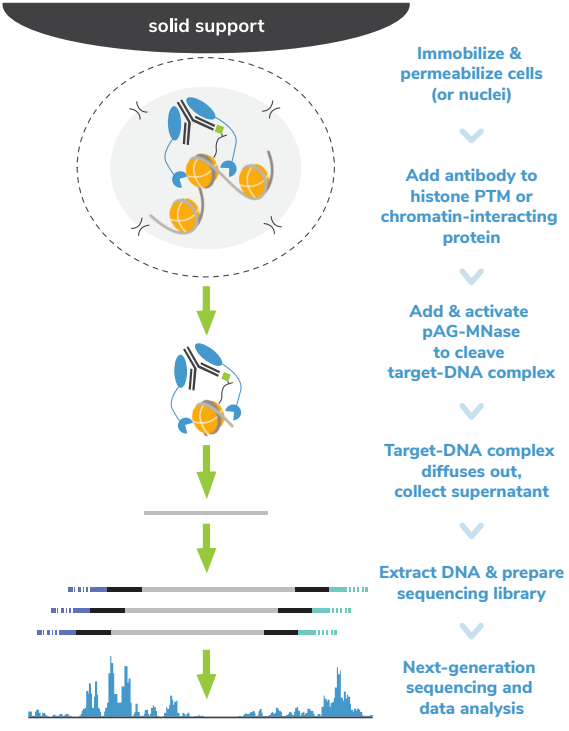

FIGURE 1

Overview of the CUTANA™ CUT&RUN protocol.

Background and Description

In CUT&RUN, purification and concentration of partitioned chromatin is an essential step that requires a workflow specifically compatible with low starting material. Utilizing specially designed tapered cleanup columns, this kit produces purified, concentrated DNA ready for library prep and next-generation sequencing (Figure 2). This enables genome-wide profiles of histone post-translational modifications (PTMs) and chromatin-associated proteins with exquisite sensitivity.

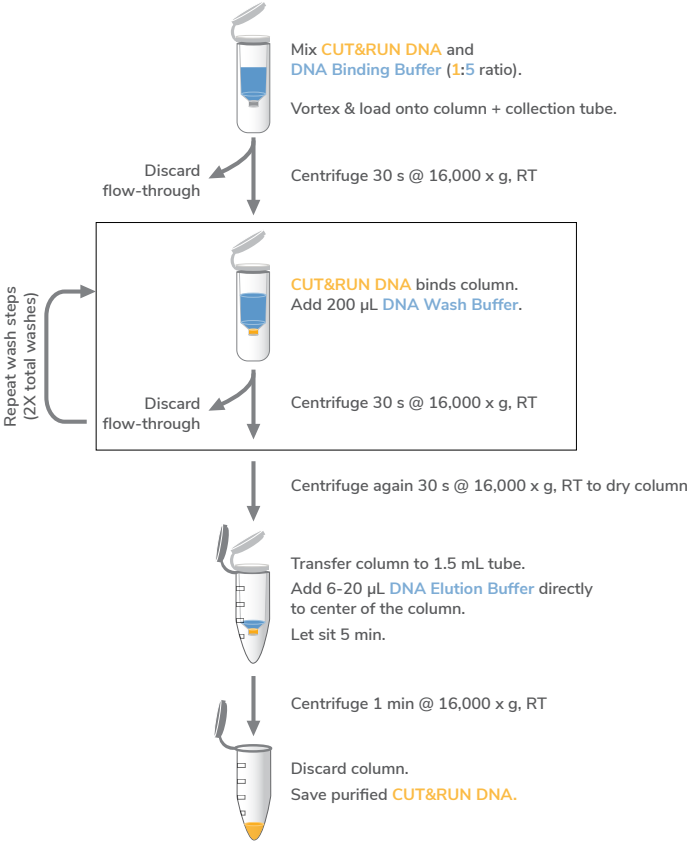

FIGURE 2

Overview of the CUTANA™ DNA Purification Kit.

Store at room temperature (RT) upon receipt:

Item	Catalog No.	Notes before use
DNA Cleanup Columns	10-0010	Use with the DNA Collection Tubes.
DNA Collection Tubes	10-0011	Use with the DNA Cleanup Columns.
DNA Binding Buffer	21-1008	Before first use add 6.9 mL isopropanol. **WARNING:** Contains toxic ingredients (see **Appendix II**).
DNA Wash Buffer	21-1009	Before use, add 20 mL ≥ 95% ethanol.
DNA Elution Buffer	21-1010	Recover final CUT&RUN DNA in 6 – 20 µL depending on desired final concentration.

Materials Required But Not Supplied

*NOTE: The kit contains sufficient materials to purify DNA from 50 CUT&RUN reactions. Additional materials will need to be procured in order to fully perform the protocol.

MATERIALS:

- 1.5 mL RNase/DNase free tubes
- Isopropanol
- ≥ 95% Ethanol

EQUIPMENT:

- Benchtop centrifuge
- Vortex (e.g. Vortex-Genie®, Scientific Industries SI-0236)
- Qubit™ 1X dsDNA HS Assay Kit (Invitrogen Q33230)
- Qubit™ 4 Fluorometer (Invitrogen Q33238) or previous version

Experimental Protocol (~30 minutes)

This kit has been optimized for use in the CUTANA™ CUT&RUN protocol, found at epicypher.com/protocols.

*NOTE: The **DNA Cleanup Columns** will retain fragments >50 bp. No specific modifications to this protocol are needed for transcription factor binding studies. However, recommendations for enrichment of small fragments during library prep are described in the CUTANA CUT&RUN protocol.

*IMPORTANT: Take care throughout the protocol - do NOT touch the pipette tip to column.

1. Prior to first use, add 6.9 mL isopropanol to **DNA Binding Buffer**.
2. Add **DNA Binding Buffer** to the CUT&RUN DNA sample at a 5:1 ratio of buffer:DNA. Mix well by vortexing.

   *NOTE: When following the CUTANA CUT&RUN protocol, targeted chromatin digestion and release yields ~84 µL of supernatant. Add 420 µL of **DNA Binding Buffer** (5:1 ratio).
3. Place a **DNA Cleanup Column** into a **DNA Collection Tube**. Load DNA sample onto a column and label the cap.
4. Centrifuge at 16,000 x g, 30 sec, at room temperature (RT). Discard flow-through and place the column back into the same collection tube.

   *NOTE: A vacuum manifold can be used in place of centrifugation. For each step, add the indicated buffer, turn the vacuum on, and allow the solution to pass through the column before turning the vacuum off.
5. Prior to first use, add 20 mL ≥ 95% ethanol to **DNA Wash Buffer**.
6. Add 200 µL **DNA Wash Buffer** to the column.
7. Centrifuge at 16,000 x g, 30 sec, RT. Discard flow-through and place the column back into the same collection tube.
8. Repeat for a total of two washes. Discard flow-through and place the column back into the same collection tube.

9. Centrifuge one additional time at 16,000 x g, 30 sec to completely dry the column.

10. Carefully transfer the column to a clean pre-labeled 1.5 mL tube, ensuring it does not contact flow-through.

11. Elute DNA by carefully adding 12 µL **DNA Elution Buffer** to center of the column without touching it and avoiding column walls. Tap the column + 1.5 mL tube on the benchtop to ensure all droplets are absorbed onto the resin.

*NOTE: 12 µL is recommended. However, DNA can be eluted in 6 – 20 µL volumes depending on anticipated yield and desired final concentration. Larger elution volumes, longer incubation times, and/or multiple rounds of elution may improve DNA yield. However, sample concentration will be reduced with larger total elution volume.

12. Let column sit 5 minutes on benchtop.

13. Centrifuge at 16,000 x g, 1 minute, RT.

14. Vortex eluted material and quick spin to collect liquid. Take 1 µL to quantify the CUT&RUN enriched DNA using the Qubit™ fluorometer as per manufacturer's instructions. See **Quality Control Checks** for typical CUT&RUN DNA yields.

15. CUT&RUN DNA can be stored at -20°C for future processing or prepped immediately for sequencing (e.g. using the CUTANA™ CUT&RUN Library Prep Kit, available at epicypher.com/14-1001).

*IMPORTANT: Although it may be tempting to analyze fragment size distribution prior to library prep, CUT&RUN enriched DNA is often below the limit of detection for such approaches. Instead, proceed directly to library prep. See **FAQs** for a discussion on the use of qPCR and Agilent TapeStation® or Bioanalyzer® to assess raw CUT&RUN DNA.

Quality Control Checks

CUT&RUN DNA yields are influenced by various factors, including starting number and type of cells, antibody specificity and efficiency, epitope abundance, and experimental perturbations. Therefore, **DNA yields from experimental reactions should not be used as a definitive metric of success.**

The best indicator of CUT&RUN success prior to library prep is that yields from the H3K4me3 positive control and target(s) of interest are greater than the IgG negative control, even if slightly so. For low cell numbers or primary cells, these differences may not be observed; however, good quality sequencing data can still be obtained.

In general, low abundance targets (e.g. H3K4me3) show small differences in yields compared to IgG, while high abundance targets (e.g. H3K27me3) display a more pronounced increase. Typical results generated using 500,000 K562 cells are shown:

Target	Target Abundance in Cells	Antibody ID	Typical Yield*
IgG (Negative Control)	None	EpiCypher 13-0042	~2-5 ng
H3K4me3 (Positive Control)	Low	EpiCypher 13-0041	~5-10 ng
H3K27me3	High	Thermo Fisher MA5-11198	~20-50 ng

*NOTE: Yields shown are from 500,000 native K562 cells and are prior to library prep.

After confirming that CUT&RUN DNA yields are in the expected range, proceed directly to library prep (e.g. using the CUTANA™ CUT&RUN Library Prep Kit, EpiCypher 14-1001 & 14-1002). DO NOT assess fragment size distribution prior to library prep, as the amount of CUT&RUN DNA recovered is likely below the limit of detection for these approaches (see **FAQs**).

Instead, fragment size distribution should be confirmed after library prep. Agilent Bioanalyzer® or TapeStation® traces should show enrichment of mononucleosome sized fragments (~300 bp = ~170 bp excised DNA + 125 bp sequencing adapters).

1. My CUT&RUN DNA yield is very low. What should I do?

5 ng of CUT&RUN DNA is recommended for library prep. To troubleshoot low yields:

- Make sure your CUT&RUN assay is fully optimized. Optimize conditions using 500,000 cells per reaction and positive and negative control antibodies (e.g. H3K4me3, EpiCypher 13-0041 & Rabbit IgG, EpiCypher 13-0042). If positive controls work (*i.e.* H3K4me3 positive control has yields slightly higher than IgG) but experimental targets show low yield, we recommend testing alternate antibodies. Note that a good ChIP-seq antibody does not guarantee success in CUT&RUN.
- Ensure that Elution Buffer is added directly to the center of the column (without disturbing the column). Let the column sit for 5 minutes before centrifugation.
- For suggestions related to CUT&RUN steps before DNA purification, see the EpiCypher CUT&RUN protocol at epicypher.com/protocols. Be sure to review "Experimental Design & Protocol Notes" and "Frequently Asked Questions." Alternatively, the CUTANA™ ChIC/CUT&RUN Kit (EpiCypher 14-1048) provides a validated set of reagents and controls for successful CUT&RUN assays.
- Some low-abundance targets (e.g. H3K4me3) may not yield 5 ng DNA from a single CUT&RUN reaction. However, high quality libraries can still be prepared. In these cases, it is recommended to use as much DNA as possible and use a library prep kit optimized for CUT&RUN. The CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001 & 14-1002) generates robust libraries using as few as 0.5 ng CUT&RUN DNA.

2. Can I assess purified CUT&RUN DNA using capillary electrophoresis or qPCR?

In ChIP protocols it is standard practice to assess fragment size distribution of isolated ChIP DNA by capillary electrophoresis (e.g. Agilent Bioanalyzer® or TapeStation®) and enrichment of known targets by qPCR. However, these strategies are not robust indicators of CUT&RUN success. Electrophoretic analysis of CUT&RUN DNA prior to library prep is NOT recommended, since the amount of DNA is often below the sensitivity of these approaches. Furthermore, because CUT&RUN is performed *in situ*, there is no chromatin input, complicating enrichment analysis by qPCR. Instead, we suggest comparing yields from positive and negative control reactions, and proceeding directly to library prep.

Appendix II: Safety Datasheet

EpiCypher, Inc.

6 Davis Dr. Ste 755
Durham, NC 27709 USA

Phone: 1-855-374-2461
Fax: 1-855-420-6111
Email: info@epicypher.com

www.epicypher.com

24 Hour Emergency Phone Number:

US & Canada: 1-800-535-5053

International: 1-352-323-3500

Product Identification

Product Name: CUTANA DNA Purification Kit
Synonyms: None.
Molecular Weight: Not applicable to mixtures.
Chemical Formula: Not applicable to mixtures.
Recommended Use: This product is for research and development only.

Component Name	Hazardous Ingredients
DNA Binding Buffer	Yes
DNA Wash Buffer	NA
DNA Elution Buffer	NA

Hazardous Identification

DNA Binding Buffer

Classification

This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Label Elements

Acute toxicity – Oral Category 4
Skin corrosion/irritations - Category 2
Serious eye damage/eye irritation - Category 2A

Hazardous Identification

Signal Word

WARNING

Hazard Statements

Harmful if swallowed, causes skin irritation, causes serious eye irritation.

Precautionary Statements

Store in a well-ventilated place. Keep container tightly closed.

Prevention

- Wear protective gloves/protective clothing/eye protection/face protection.
- Wash face, hands and any exposed skin thoroughly after handling.
- Do not eat, drink or smoke when using this product.

Response

- **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **If eye irritation persists:** Get medical advice/attention.
- **IF ON SKIN (OR HAIR):** Take off immediately all contaminated clothing. Rinse skin with water/shower. Wash contaminated clothing before reuse. **If skin irritation occurs:** Get medical advice/attention.
- **IF SWALLOWED:** Call a POISON CENTER or doctor if you feel unwell. Rinse mouth.

Composition and Information on Ingredients

DNA Binding Buffer (mixture)

Chemical Name	Kit Volume	CAS Number
Trade secret	<10 mL	*

* The exact percentage (concentration) of composition has been withheld as a trade secret.

First Aid Measures

General advice: Show this safety data sheet to the doctor in attendence.

Inhalation: Get medical attention immediately if symptoms occur. Remove to fresh air.

Eye contact: Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Keep eye wide open while rinsing. Do not rub affected area. Get medical attention if irritation develops and persists.

Skin contact: Wash off immediately with soap and plenty of water for at least 15 minutes. Get medical attention if irritation develops and persists.

Ingestion: Do NOT induce vomiting. Clean mouth with water and drink afterwards plenty of water. Never give anything by mouth to an unconscious person. If symptoms persist, call a physician.

Most important symptoms and effects, both acute and delayed: Burning sensation. May cause redness and tearing of the eyes.

Self protection of the first aider: Avoid contact with skin, eyes, or clothing. Wear personal protective clothing (see **Exposure Controls/PPE**).

Note to Physicians: Treat symptomatically.

Fire Fighting Measures

Suitable Extinguishing Media: Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.

Unsuitable Extinguishing Media: CAUTION: Use of water spray when fighting fire may be inefficient.

Specific hazards arising from chemical: No information available.

Explosion data: Sensitivity to mechanical or static discharge: None.

Special protective equipment for fire-fighters: Fire-fighters should wear self-contained breathing apparatus and full firefighting turnout gear. Use personal protective equipment.

Accidental Release Measures

Spill response: Prevent further leakage if it is safe to do so. Handle in accordance with good industrial hygiene and safety practice. Ensure adequate ventilation and avoid contact with skin, eyes, or clothing. Use personal protective equipment as required. Pick up and transfer waste to properly labeled containers.

Waste disposal method: Dispose of in accordance with all federal, state and local regulations. Dispose of waste in accordance with environmental legislation.

Contaminated packaging: Do not reuse empty containers.

Handling and Storage

Safe Handling: Use personal protective equipment. Avoid contact with skin, eyes or clothing. Take off contaminated clothing and wash before reuse. Do not eat, drink, or smoke when using this product.

Storage Conditions: Keep out of the reach of children. Keep containers tightly closed in a dry, cool and well-ventilated place. Keep in properly labeled containers. Store in accordance with local regulations.

Exposure Controls / PPE

Exposure limits: The following ingredients are the only ingredients of the product above the cut-off level which have an exposure limit applicable in the region for which this safety data sheet is intended or other recommended limit. At this time, the other relevant constituents have no known exposure limits from the sources listed here.

Other information: Vacated limits revoked by the Court of Appeals decision in AFL-CIO v. OSHA, 965 F.2d 962 (11th Cir., 1992).

Engineering Controls: Showers, eyewash stations, ventilation systems.

Exposure Controls / PPE, Continued

Personal Protective Equipment

Eye/face protection: If splashes are likely to occur, wear safety glasses with side-shields.

Hand protection: Wear suitable impervious gloves.

Skin and body protection: Wear suitable protective clothing.

Respiratory protection: No protective equipment is needed under normal use conditions. If exposure limits are exceeded or irritation is experienced, ventilation and evacuation may be required.

General hygiene considerations: Avoid contact with skin, eyes, or clothing. Wear suitable gloves and eye/face protection. Do not eat, drink or smoke when using this product.

Physical and Chemical Properties

Appearance:	Colorless Liquid
Odor:	None
Boiling Point:	No data available
Melting Point:	No data available
Solubility:	No data available
Flash Point:	No data available
Specific Gravity:	No data available
pH:	5.10-5.30

Stability and Reactivity

Chemical Stability: Stable under ordinary conditions of use and storage.

Incompatibilities: Strong oxidizers, strong acids and bases.

Conditions to Avoid: None known based on information supplied.

Hazardous Decomposition Products: None known based on information supplied.

Toxicological Information

Inhalation: Specific test data for the substance or mixture is not available. May cause irritation of respiratory tract (based on components).

Eye contact: Irritating to eyes. Specific test data for the substance or mixture is not available. Causes serious eye irritation (based on components).

Skin contact: Causes skin irritation (based on components). Specific test data for the substance or mixture is not available.

Ingestion: Specific test data for the substance or mixture is not available. Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea. Harmful if swallowed (based on components).

Symptoms related to the physical, chemical and toxicological characteristics: Redness. May cause redness and tearing of the eyes.

Acute toxicity: Numerical measures of toxicity. The following values are calculated based on chapter 3.1 of the GHS document:
ATEmix (oral) 1,217.00 mg/kg; ATEmix (dermal) 5,124.00 mg/kg.

Carcinogenicity: No information available.

Target organ effects: Eyes, skin.

Ecological Information

The environmental impact of this product has not been fully investigated.

Pesistence and degradability: No information available.

Bioaccumulation: There is no data for this product.

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).

24 Hour Emergency Phone Number:

US & Canada: 1-800-535-5053

International: 1-352-323-3500

EpiCypher, Inc.
6 Davis Dr. Ste 755
Durham, NC 27709 USA

www.epicypher.com
Ph: 1-855-374-2461 F: 1-855-420-6111
Email: info@epicypher.com Tech Support: techsupport@epicypher.com