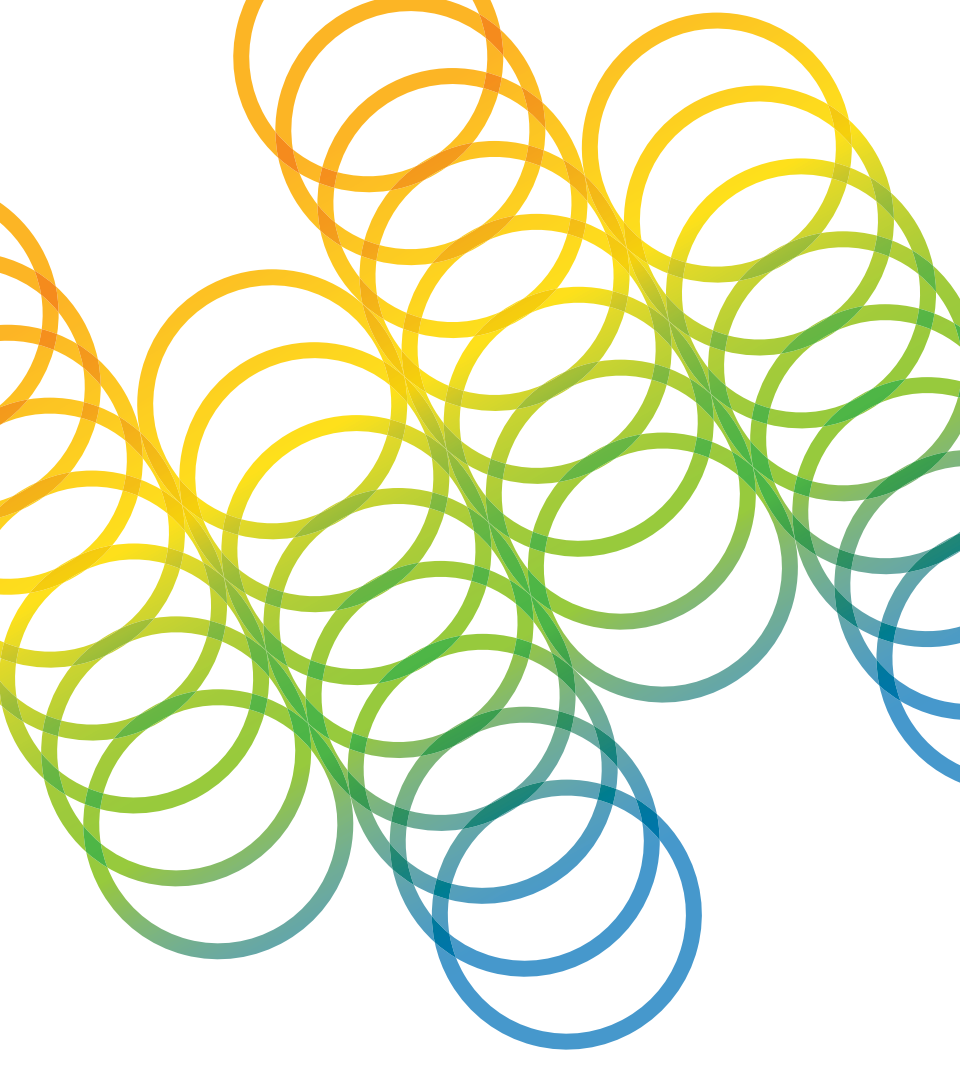

EpiCypher®
Bringing Epigenetics to Life

CUTANA™
DNA Purification Kit
Version 1.0

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

Kit Version 1.0
User Manual Version 1.0

EpiCypher®

CUTANA™

DNA Purification Kit

Catalog No. 14-0050
50 Samples

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 input and sequencing depth[3].

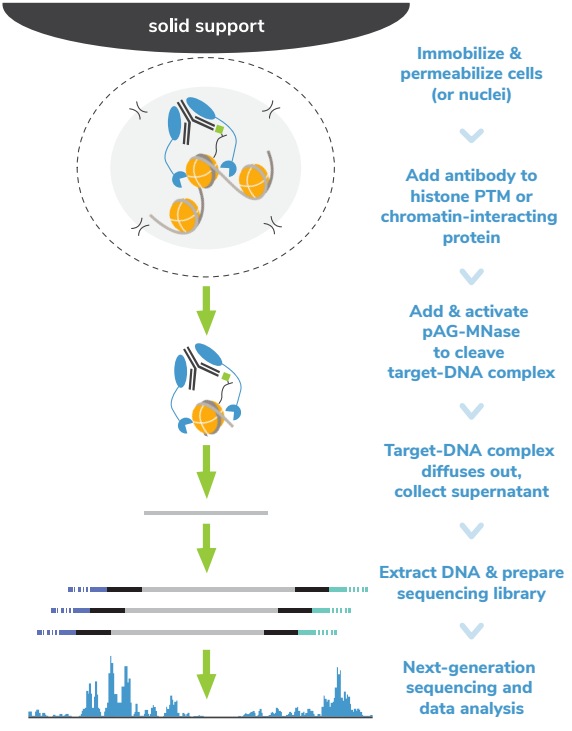

FIGURE 1

Overview of the CUTANA CUT&RUN protocol.

Background and Description

In CUT&RUN, high yield purification and concentration of partitioned chromatin is an essential step; one 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 preparation and next-generation sequencing (Figure 2). This enables genome-wide profiles of histone post-translational modifications (PTMs) and chromatin-associated proteins with exquisite sensitivity.

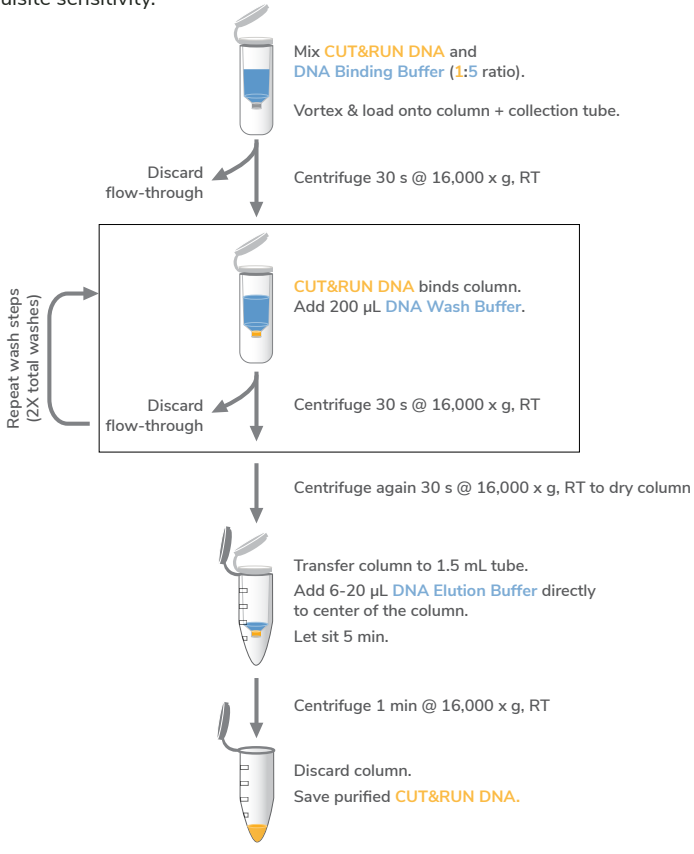

FIGURE 2

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	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 samples. Additional materials will need to be procured in order to fully perform the protocol.*

MATERIALS:

- 1.5 mL RNase/DNase free tubes

EQUIPMENT:

- Benchtop centrifuge
- Vortex (e.g. Scientific Industries Vortex-Genie #SI-0236)
- Qubit™ 1X dsDNA HS Assay Kit (Invitrogen #Q33230)
- Qubit™ 4 Fluorometer (Invitrogen #Q33226)

Experimental Protocol (~30 minutes)

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

*NOTE: *The DNA Cleanup Columns will retain fragments > 50 bp. No specific modifications to this protocol are needed for TF binding studies (e.g. resolution of TF footprinting), however additional modifications during the library preparation steps are described in the CUT&RUN protocol.*

*NOTE: *Take care throughout the protocol not to touch the pipette tip to the column.*

1. Add **DNA Binding Buffer** to each CUT&RUN DNA sample at a 5:1 ratio of buffer:DNA. Mix well by vortexing.

   *NOTE: *When following the EpiCypher CUTANA CUT&RUN protocol, the quenched chromatin digest reaction separated from the magnetic beads produces ~84 µL of supernatant. Add 420 µL of DNA Binding Buffer (5:1 ratio).*

2. For every sample, place a **DNA Cleanup Column** into a **Collection Tube**. Load each sample onto a column and label the top.
3. Centrifuge for 30 sec at 16,000 x g, RT. Discard the flow-through. Place the collection tube back on to the column.

   *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.*

4. Prior to first use, add 20 mL ≥ 95% ethanol to **DNA Wash Buffer**.
5. Add 200 µL **DNA Wash Buffer** to each sample column.
6. Centrifuge for 30 sec at 16,000 x g, RT. Discard the flow-through. Place the collection tube back on to the column.
7. Repeat for a total of two washes.
8. Remove flow-through. Centrifuge one additional time for 30 sec at 16,000 x g to completely dry the column.
9. Transfer column to a clean pre-labeled 1.5 mL microfuge tube, ensuring the column does not come into contact with the flow-through.

10. Elute DNA by adding 12 µL DNA Elution Buffer, taking care to ensure the buffer is added to the center of the column rather than the wall. Tap the column + collection 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.*

11. Let sit 5 minutes, then centrifuge for 1 minute at 16,000 x g, RT.
12. Vortex eluted material and use 1 µL to quantify the CUT&RUN-enriched DNA using the Qubit fluorometer as per manufacturer's instructions. See "**Quality Control Checks**" for typical DNA yields.
13. CUT&RUN DNA can be stored at -20°C for future processing or prepped immediately for sequencing (e.g. using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina or equivalent approach).

*IMPORTANT: *In ChIP protocols, it is common practice to analyze isolated ChIP DNA using agarose gel or capillary electrophoresis to assess fragment size distribution prior to library preparation. However, electrophoretic analysis of CUT&RUN DNA prior to library preparation is NOT recommended, since the amount of DNA is likely to be below the sensitivity of detection for these approaches. Instead, proceed directly to library preparation.*

Quality Control Checks

The yield of CUT&RUN enriched DNA can vary in different experimental settings and for different antibodies, even for antibodies to the same target. Therefore, **DNA yield should not be used as a definitive metric of experimental success**. However, in general the yield from the positive control antibody (e.g. H3K4me3 or H3K27me3, see Table below) should be slightly higher than the IgG negative control. Typical results generated using 500,000 K562 cells are shown:

Target	Target Abundance in Cells	Antibody ID	Typical Yield* (ng DNA)
IgG (Negative Control)	None	EpiCypher #13-0042	~2-5
H3K4me3 (Positive Control)	Low	EpiCypher #13-0041	~5-10
H3K27me3	High	EpiCypher #13-0030	~20-50

*NOTE: *Total yields are influenced by a number of considerations, including antibody specificity, antibody efficiency, and epitope abundance in each cell type. Yields shown are from 500,000 native K562 cells and are prior to PCR amplification.*

After confirming that CUT&RUN DNA yields are in the expected range, proceed directly to preparation of Illumina sequencing libraries (e.g. using NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®, New England Biolabs Catalog No. E7645S or equivalent approach). DO NOT attempt to assess fragment size distribution prior to library amplifcation, as the amount of CUT&RUN DNA recovered is likely to be below the limit of detection for agarose gel and/or capillary electrophoresis (e.g. Agilent Bioanalyzer™ or Tapestation™). After library preparation, confirm that CUT&RUN resulted in enrichment of primarily mononucleosome fragments (~150 bp + sequencing adapters).

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

While a minimum of 5-10 ng DNA is typically recommended for library preparation, high quality CUT&RUN libraries can be prepared even when DNA yield is below Qubit limit of detection. To gain confidence with CUT&RUN, first optimize conditions using 500,000 cells and positive and negative control antibodies (e.g. EpiCypher H3K4me3, Catalog No. 13-0041 and Rabbit IgG, Catalog No. 13-0042). If positive controls worked (e.g. H3K4me3 yield is slightly higher than IgG) but other targets produced low yield, try alternate antibodies. Note that a good ChIP-seq antibody does not guarantee success in CUT&RUN.

For troubleshooting low yield, also ensure that the Elution Buffer is added directly to the center of the column (without disturbing the column itself) and that the column is allowed to sit for 5 minutes before centrifugation. For additional suggestions related to the CUT&RUN workflow prior to DNA Purification, see the full EpiCypher CUT&RUN protocol at EpiCypher.com/resources/protocols. Take particular note of "Protocol Notes", "Critical Steps", and "Frequently Asked Questions". Alternatively, the CUTANA ChIC CUT&RUN Kit (EpiCypher Catalog No. 14-1048) provides a validated set of reagents and controls to help ensure success in CUT&RUN.

2. My CUT&RUN DNA does not show predominant enrichment of mononucleosomes. What went wrong?

See question above for critical controls and key notes. Importantly, ensure that pAG-MNase digestion conditions are optimized.

3. I don't see anything when I run my CUT&RUN DNA on an agarose gel/ Bioanalyzer/Tapestation?

Since CUT&RUN has lower background and is compatible with fewer cells compared to ChIP-seq, it is not recommended to assess fragment size distribution using agarose gel or capillary electrophoresis prior to library preparation. Such analysis is not indicative of the success of a CUT&RUN experiment, as the amount of DNA recovered is often below the sensitivity of detection for these approaches. Instead, assess DNA yield compared to positive (e.g. H3K4me3) and negative (IgG) controls, and determine fragment size distribution of sequence-ready libraries.

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 2
Flammable liquids - Category 3

Hazardous Identification

Signal Word

WARNING

Hazard Statements

Harmful if swallowed, causes skin irritation, causes serious eye irritation, flammable liquid and vapor.

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.
- Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
- Ground/bond container and receiving equipment.
- Use only non-sparking tools.
- Take precautionary measures against static discharge.

Response

- **IF EXPOSED OR CONCERNED:** Get medical advice/attention.
- **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 skin irritation occurs: 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 SWALLOWED:** Call a POISON CENTER or doctor if you feel unwell. Rinse mouth.
- **IN CASE OF FIRE:** Use CO_2, dry chemical, or foam to extinguish.

Composition and Information on Ingredients

DNA Binding Buffer (mixture)

Chemical Name	Kit Volume	CAS Number
Isopropyl Alcohol	< 11 ml	67-63-0
Sodium Perchlorate	< 2 ml	7601-89-0
Guanidine Hydrochloride	< 8 ml	50-01-1

First Aid Measures

General advice: If symptoms persist, call a physician. Do not breathe dust/fume/gas/mist/vapors/spray. Do not get in eyes, on skin, or on clothing.

Inhalation: Remove to fresh air. If breathing is irregular or stopped, administer artificial respiration. Avoid direct contact with skin. Use barrier to give mouth-to-mouth resuscitation. If symptoms persist, call a physician.

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

Skin contact: Wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes. Wash contaminated clothing before reuse. If skin irritation persists, call a physician. Immediate medical attention is not required.

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: May cause redness and tearing of the eyes. Prolonged contact may cause redness and irritation. Vapors may cause drowsiness and dizziness.

Note to Physicians: Treat symptomatically.

Fire Fighting Measures

Suitable Extinguishing Media: Dry chemical. Carbon dioxide (CO_2). Water spray. Alcohol resistant foam.

Specific hazards arising from chemical: Risk of ignition. Keep product and empty container away from heat and sources of ignition. In the event of fire, cool tanks with water spray. Fire residues and contaminated fire extinguishing water must be disposed of in accordance with local regulations.

Special protective equipment for fire-fighters: In the event of a fire, wear full protective clothing and NIOSH-approved self-contained breathing apparatus with full facepiece operated in the pressure demand or other positive pressure mode.

Accidental Release Measures

Spill response: Absorb or cover with dry earth, sand or other non-combustible material and transfer to containers. Soak up condensate or spills with inert absorbent material and collect in ventilated waste container for disposal. Wear lab coat, chemical resistant gloves and safety glasses. Take precautionary measures against static discharges. Wash down the spill site.

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

Handling and Storage

Safe Handling: Use personal protection equipment. Avoid breathing vapors or mists. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. Use grounding and bonding connection when transferring this material to prevent static discharge, fire or explosion. Use with local exhaust ventilation. Use sparkproof tools and explosion-proof equipment. Keep in an area equipped with sprinklers. Avoid contact with skin, eyes or clothing. Take off contaminated clothing and wash before reuse.

Storage Conditions: Keep containers tightly closed in a dry, cool and well-ventilated place. Keep away from heat, sparks, flame and other sources of ignition. Keep in properly labeled containers. Do not store near combustible materials. Keep in an area equipped with sprinklers. 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.

Isopropyl Alcohol 67-63-0	STEL: 400 ppm TWA: 200 ppm	TWA: 400 ppm TWA: 980 mg/m3 (vacated) TWA: 400 ppm (vacated) TWA: 980 mg/m3 (vacated) STEL: 500 ppm (vacated) STEL: 1225 mg/m3	IDLH: 2000 ppm TWA: 400 ppm TWA: 980 mg/m3 STEL: 500 ppm STEL: 1225 mg/m3

Engineering Controls: Showers, eyewash stations, ventilation systems.

Personal Protective Equipment

Eye/face protection: Tight sealing safety goggles.

Hand protection: Wear suitable impervious gloves.

Skin and body protection: Wear suitable protective clothing, long sleeved clothing, Chemical resistant apron, and antistatic boots.

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.

Physical and Chemical Properties

Appearance:	Colorless Liquid	Solubility:	No data available
Odor:	Alcohol	Flash Point:	27 °C / 80.6 °F
Boiling Point:	90 °C / 194 °F	Specific Gravity:	No data available
Melting Point:	No data available	pH:	5.10-5.30

Stability and Reactivity

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

Hazardous Decomposition Products: None known based on information supplied.

Incompatibilities: Strong oxidizers, strong acids and bases.

Conditions to Avoid: Heat, flame, sparks, incompatibles.

Toxicological Information

Inhalation Specific test data for the substance or mixture is not available. May cause irritation of respiratory tract.

Eye contact Specific test data for the substance or mixture is not available. Irritating to eyes. Causes serious eye irritation.

Skin contact Specific test data for the substance or mixture is not available. Prolonged contact may cause redness and irritation. Repeated exposure may cause skin dryness or cracking.

Ingestion Specific test data for the substance or mixture is not available. Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea. Harmful if swallowed. May cause drowsiness or dizziness.

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) 1,127.90 mg/kg ATEmix (dermal) 4,599.60 mg/kg ATEmix (inhalation-dust/mist) 157.80 mg/

Carcinogenicity. Isopropyl Alcohol IARC Group 3

Target organ effects Respiratory system, Eyes, Skin.

Ecological Information

Chemical Name	Aquatic Plants	Fish	Crustacea
Isopropyl Alcohol 67-63-0	EC50: > 1000 mg/L (72h, Desmodesmus subspicatus) EC50: > 1000 mg/L (96h, Desmodesmus subspicatus)	LC50: =9640 mg/L (96h, Pimephales promelas) LC50: > 1400000 µg/L (96h, Lepomis macrochirus) LC50: =11130mg/L (96h, Pimepha les promelas.	EC50: =13299mg/L (48h, Daphnia magna)
Guanidine Hydrochloride 50-01-1	NA	LC50: =1758mg/L (48h, Leucisc usidus)	NA

Disposal Information

Dispose in accordance with local, state, and federal regulations.

US EPA Waste Number D001

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).
4. Shah et *al.* Examining the roles of H3K4 methylation states with systematically characterized antibodies. **Mol. Cell** 72, 162-177 (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