EpiCypher®

# CUTANA™ GST-MeCP2 for meCUT&RUN

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| **Catalog No** | 15-2002-S | **Species** | Human |
| **Lot No** | 24318001-86 | **Source** | *E. coli* |
| **Pack Size** | 24 Reactions / 60 µL | **Epitope Tag** | GST, 6xHis |
| **Concentration** | 20X | **MW** | 39.1 kDa |

## DESCRIPTION

CUTANA™ GST-MeCP2 for meCUT&RUN is a GST-tagged MeCP2 methyl binding domain that is validated to enrich regions of chromatin with symmetrically methylated CpGs in CUTANA™ meCUT&RUN (EpiCypher Kit 14-1060-24), a modified CUT&RUN workflow that enables streamlined, high-resolution mapping of DNA methylation. meCUT&RUN can be followed by a traditional library prep method, such as the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001/14-1002), to provide ~150 bp resolution profiles of DNA methylation enrichment, or by a cytosine conversion strategy such as Enzymatic Methyl-seq (NEB® EM-seq™, preferred) or bisulfite sequencing to provide base-pair resolution of 5-methylcytosine (5mC).

## RECOMMENDED ACCESSORY PRODUCTS

| Item | Cat No | Item | Cat No |
|---|---|---|---|
| CUTANA™ Anti-GST Tag Antibody | 13-0073 | CUTANA™ ChIC/CUT&RUN Kit | 14-1048 |
| CUTANA™ CUT&RUN Library Prep Kit | 14-1001/14-1002 | | |

## TECHNICAL INFORMATION

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| **Storage** | Stable for 12 months at -20°C from date of receipt. The protein is not subject to freeze/thaw under these conditions. |
| **Formulation** | 25 mM HEPES-NaOH pH 6.5, 200 mM NaCl, 1 mM DTT, 50% glycerol, 2 mM $MgCl_2$ |

## APPLICATION NOTES

To perform meCUT&RUN with this reagent and a CUTANA CUT&RUN Kit (EpiCypher 14-1048), please follow the meCUT&RUN Kit Manual. If using homemade buffers, adjust the EpiCypher CUT&RUN Protocol as follows:
The Manual and the Protocol can be found at epicypher.com/protocols.

**Day 1**:

- Prepare Pre-Wash Buffer with 200 mM NaCl*. This is used to prepare Wash Buffer, Digitonin Buffer, and Antibody Buffer.
  **NOTE: Standard CUT&RUN uses 150 mM NaCl in these buffers; however, excess salt is recommended to improve MeCP2 specificity for methylated DNA.*
- In place of a primary antibody, add 2.5 µL of GST-MeCP2 to each reaction, and incubate on a nutator at 4˚C overnight.

**Day 2**:

- On Day 2, discard the GST-MeCP2 supernatant and resuspend each reaction in 50 µL cold Digitonin Buffer.
- Add 1.0 µL anti-GST Tag Antibody (EpiCypher 13-0073)* to each reaction and incubate on a nutator at room temperature for 30 minutes.
  **NOTE: Secondary-only (anti-GST Tag) reactions are recommended as a negative control.*
- At the end of the incubation, discard the supernatant and wash two times with 200 µL cold Digitonin Buffer.
- Resuspend in 50 µL per reaction cold Digitonin Buffer and proceed to pAG-MNase (EpiCypher 15-1016/15-1116) addition per the standard protocol.

*Application Notes continued on next page*

## APPLICATION NOTES (CONT.)

**Sequencing Libraries can be prepared using two methods**:
• Option 1: Traditional library prep, which provides a CUT&RUN-like view of genomic regions with high DNA methylation. The CUTANA CUT&RUN Library Prep Kits (EpiCypher 14-1001/14-1002) are compatible with this approach.
• Option 2: A cytosine conversion strategy (e.g., New England Biolabs NEBNext® Enzymatic Methyl-seq v2 Kit, E8015), which provides base-pair resolution of 5mC enrichment. Prior to EM-seq conversion, add CUTANA™ Fragmented Controls for DNA Methylation Sequencing (EpiCypher 14-1804) to each reaction as outlined in the 14-1804 TDS.

## VALIDATION DATA

**meCUT&RUN Methods** meCUT&RUN was performed starting with 500k K562 cells with either 2.5 µL of 20X GST-MeCP2 added as the primary binding reagent or 0.5 µg of a secondary antibody-only control (anti-GST antibody, EpiCypher 13-0073) to determine background cleavage. Library preparation was performed using 5 ng of meCUT&RUN-enriched DNA (or the total amount recovered if less than 5 ng) using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001/14-1002). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 7.7 million reads (anti-GST) and 8.4 million reads (GST-MeCP2). Data were aligned to the hg38 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

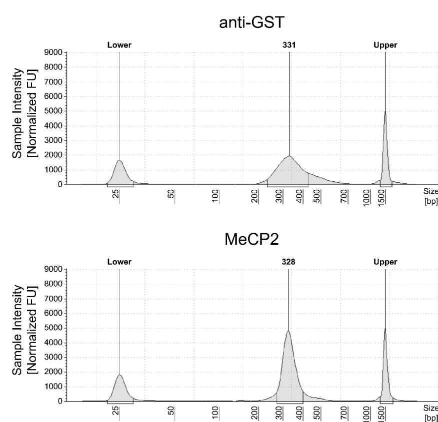


**FIGURE 1 meCUT&RUN DNA fragment size distribution analysis.** meCUT&RUN was performed as described above. Library DNA was analyzed by Agilent TapeStation®. This analysis confirmed that mononucleosomes were predominantly enriched in meCUT&RUN (~300 bp peaks represent 150 bp nucleosomes + sequencing adapters).

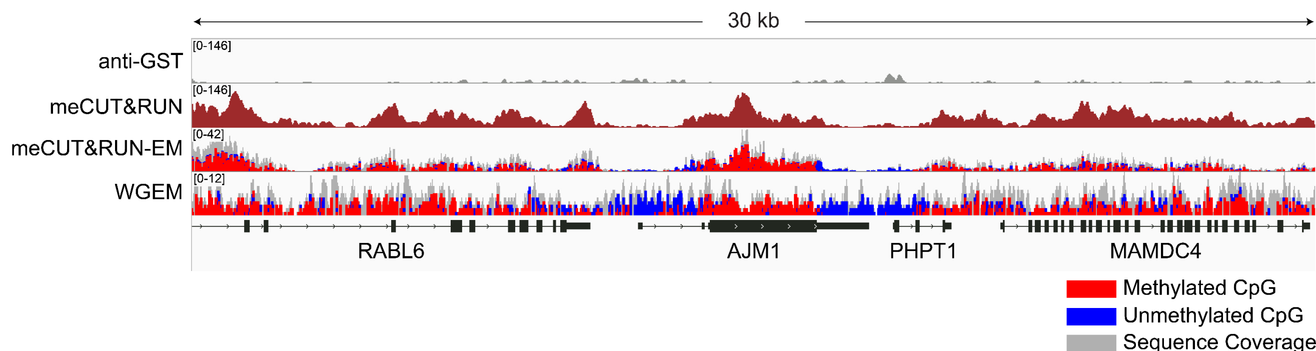


**FIGURE 2 Gene browser tracks.** meCUT&RUN was performed as described above. A 30 kb window at the AJM1 gene is shown for anti-GST antibody and meCUT&RUN. Tracks are also shown with representative data for meCUT&RUN followed by EM-seq (meCUT&RUN-EM) and whole genome EM-seq (WGEM), using the New England Biolabs NEBNext® Enzymatic Methyl-seq v2 Kit (NEB E8015). The meCUT&RUN kit produced the expected genomic distribution, showing enrichment of methylated DNA that approximates the methylated CpG pattern observed in WGEM. Images were generated using the Integrative Genomics Viewer (IGV, Broad Institute).