Estrogen Receptor Alpha (N-Terminal) CUTANA™ CUT&RUN

Catalog No	13-2011	**Type**	Polyclonal
Lot No	21085001-02	**Host**	Rabbit
Pack Size	100 µL	**Concentration**	1,000 µg/mL
Applications	CUT&RUN, IP, IHC	**Reactivity**	Human, Mouse (predicted), Rat (predicted)

DESCRIPTION

This antibody meets EpiCypher's "CUTANA Compatible" criteria for performance in Cleavage Under Targets and Release Using Nuclease (CUT&RUN) and/or Cleavage Under Targets and Tagmentation (CUT&Tag) approaches to genomic mapping. Every lot of a CUTANA Compatible antibody is tested in the indicated approach using EpiCypher optimized protocols (epicypher.com/protocols) and determined to yield peaks that show a genomic distribution pattern consistent with reported function(s) of the target protein. Estrogen Receptor Alpha N-terminal (ER alpha N-term) antibody shows CUT&RUN peaks in response to estradiol stimulation (**Figure 1**) that overlap with known estrogen response element (ERE) binding motifs (**Figure 2**). Overlap is further observed with peaks from an antibody to a different ER alpha epitope (C-term) and NCOA3 (SRC3), which co-activates ER-mediated transcription [1] (**Figure 2**).

TECHNICAL INFORMATION

Immunogen	Between amino acids 1 and 50
Storage	Stable for 1 year at 4°C from date of receipt
Formulation	Antigen affinity-purified antibody in Tris-citrate/phosphate buffer pH 7-8, 0.09% sodium azide

RECOMMENDED DILUTION

CUT&RUN	0.5 µg per reaction	**Immunoprecipitation**	3 - 5 µg/mg lysate
Immunohistochemistry	1:1,000 - 1:5,000		

Epitope retrieval with citrate buffer pH 9.0 is recommended for FFPE tissue sections

GENE & PROTEIN INFORMATION

UniProt ID	P03372
Gene Name	ESR1
Protein Name	Estrogen receptor
Target Size	66 kDa
Alternate Names	ER, ER-alpha, Estradiol receptor, Nuclear receptor subfamily 3 group A member 1, ESR, NR3A1

REFERENCES

[1] Wagner et al. *BMC Cancer* (2013). PMID: 24304549

VALIDATION DATA

CUT&RUN Methods

Serum-starved MCF7 cells were treated with estradiol (E2) or vehicle control for 45 minutes. CUT&RUN was performed on 500k cells with 0.5 µg of either ER Alpha (N-Term), ER Alpha (C-Term; EpiCypher 13-2012), NOCA3/SRC3 (EpiCypher 13-2013), H3K4me3 positive control (EpiCypher 13-0041), or IgG negative control (EpiCypher 13-0042) antibodies using the CUTANA™ ChIC/CUT&RUN Kit v2.0 (EpiCypher 14-1048). Library preparation was performed with 5 ng of DNA (or the total amount recovered if less than 5 ng) using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001/14-1002). Both kit protocols were adapted for high throughput Tecan liquid handling. Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

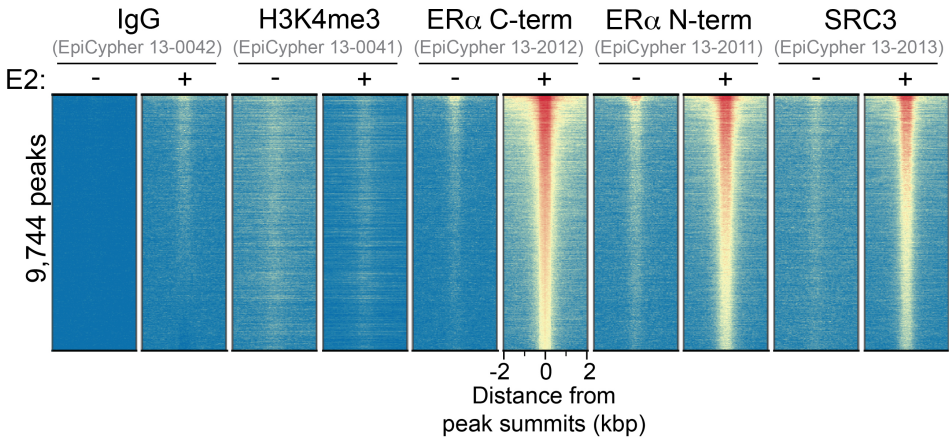

FIGURE 1 ER alpha N-term peaks in CUT&RUN. CUT&RUN was performed as described above. Heatmaps show ER alpha N-term peaks relative to IgG negative control, H3K4me3 positive control, ER alpha C-term, and SRC3 antibodies in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal.

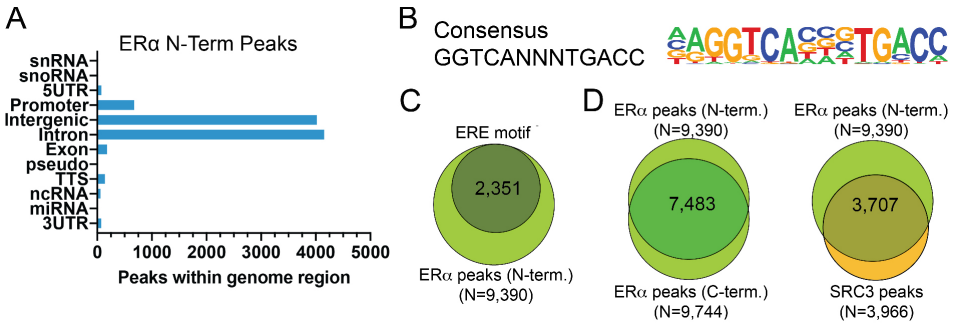

FIGURE 2 ER alpha N-term peak analysis in CUT&RUN. Peaks from the E2-treated samples in Figure 1 were called using MACS2. (**A**) The number of ER alpha N-term peaks which fall into distinct classes of functionally annotated genomic regions is plotted. (**B**) Homer analysis determined that the ERE consensus motif, represented as a sequence logo position weight matrix, was enriched under ER alpha N-term peaks. (**C**) The number of ER alpha N-term peaks containing consensus motifs from panel B is shown by Venn Diagram. (**D**) The number of ER alpha N-term peaks that overlap with ER alpha C-term and SRC3 antibodies are represented by Venn Diagram.

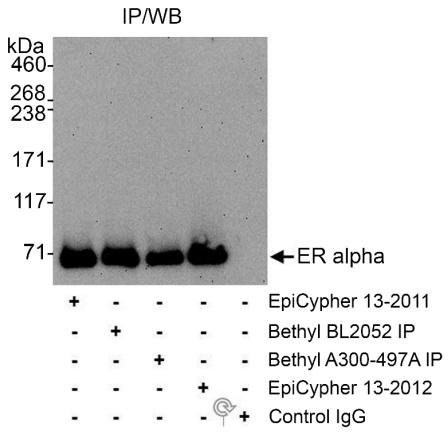

FIGURE 3 Immunoprecipitation data. EpiCypher ER alpha N-term antibody (3 µg) was used to immunoprecipitate whole cell lysates isolated from MCF7 cells (1.0 mg per IP). A negative control IgG antibody and positive control antibody to different ER alpha epitopes (EpiCypher 13-2012 and Bethyl Laboratories) were also used to demonstrate specificity of the IP. Immunoprecipitates were loaded onto a 4-8% SDS-PAGE gel (25% of IP loaded) and probed via western blot with EpiCypher 13-2012 ER alpha C-term antibody (0.1 µg/mL).

VALIDATION DATA

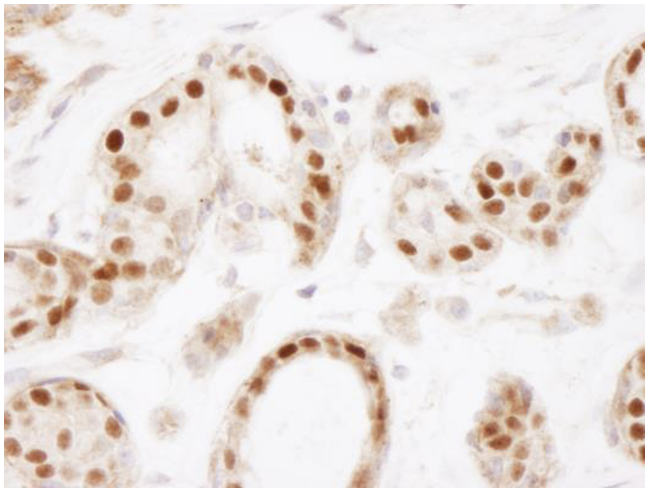

FIGURE 4 **Immunohistochemistry data.** FFPE section of human breast examined using ER alpha N-term antibody (1:2,500 dilution, 0.4 µg/mL).