abcam

Product datasheet

ChIP-Seq High Sensitivity Kit ab185908

3 References 3 Images

Overview

Product name	ChIP-Seq High Sensitivity Kit
Sample type	Tissue, Adherent cells, Suspension cells
Assay time	7h 00m
Product overview	ab185908 is a complete set of reagents required for carrying out a successful ChIP-Seq starting from mammalian cells or tissues. The kit is designed to selectively enrich a chromatin fraction containing specific DNA sequences from various species, particularly mammals, and to prepare a ChIP-Seq library for next generation sequencing using Illumina® platforms such as Illumina® Genome Analyzer II, HiSeq and MiSeq systems. The optimized protocol and components of the kit allow capture of low abundance protein/DNA complexes with minimized non-specific background levels and the ability to construct both non-barcoded (singleplexed) and barcoded (multiplexed) ChIP-Seq libraries quickly with reduced bias. **Starting Materials:** Starting materials can include various tissue or cell samples such as culture cells from a flask or plate, fresh and frozen tissues, etc. **Input Amount of Tissue/Cells:** In general, the amount of cells and tissues for each reaction can be 1 x 10^5 to 1 x 10^6 and 5 mg to 50 mg, respectively. For optimal preparation, the input amount should be 4 to 5 x 10^5cells or 20 to 30 mg tissues so that the amount of DNA enriched from the ChIP reaction can range from at least 1 ng to 100 ng. Illumina® is a registered trademark of Illumina, Inc.
Notes	Protein-DNA interaction plays a critical role for cellular functions such as signal transduction, gene transcription, chromosome segregation, DNA replication and recombination, and epigenetic silencing. Identifying the genetic targets of DNA binding proteins and knowing the mechanisms of protein-DNA interaction on a genome-wide scale is important for understanding cellular processes. Chromatin immunoprecipitation (ChIP) followed by next generation sequencing (ChIP-Seq) offers an advantageous tool for studying genome-wide protein-DNA interactions. It allows for detection that a specific protein binds to specific sequences in living cells. In particular, ChIP antibodies targeted against various transcriptional factors (TF) for genome-wide transcription factor binding site analysis by Chip-Seq is in high demand. Such analysis requires that ChIPed DNA contain minimal background for reliably identifying true TF-enriched regions. Currently used ChIP-Seq methods play an important role in identifying genome-wide protein-DNA interaction. The ChIP-Seq High Sensitivity Kit combines microplate-based ultra ChIP and high sensitive DNA library construction technologies.

Tested applications **Suitable for:** ChIP-sequencing

Properties

Storage instructions Please refer to protocols.

Components	12 tests	24 tests
1000X Protease Inhibitor Cocktail	1 x 15µl	1 x 30µl
10X End Polishing Buffer	1 x 30µl	1 x 60µl
2X HiFi PCR Master Mix	1 x 160µl	1 x 320µl
2X Ligation Buffer	1 x 250µl	1 x 500µl
8-Well Assay Strips (with Frame)	1 x 2 units	1 x 4 units
8-Well Strip Caps	1 x 2 units	1 x 4 units
Adaptors (50 µM)	1 x 15µl	1 x 30µl
Adhesive 8-Well Strip Film	1 x 4 units	1 x 8 units
Antibody Buffer	1 x 1ml	1 x 2ml
Anti-RNA Polymerase II	1 x 5µl	1 x 10µl
Blocker Solution	1 x 1ml	1 x 2ml
ChIP Buffer	1 x 6ml	1 x 12ml
DNA Binding Solution	1 x 7ml	1 x 14ml
DNA Elution Buffer	1 x 0.5ml	1 x 1ml
DNA Release Buffer	1 x 7ml	1 x 14ml
Elution Buffer	1 x 1ml	1 x 2ml
End Polishing Enhancer	1 x 13µl	1 x 26µl
End Polishing Enzyme Mix	1 x 13µl	1 x 26µl
Enrichment Enhancer	1 x 25µl	1 x 50µl
F-Collection Tube	1 x 15 units	1 x 30 units
F-Spin Column	1 x 15 units	1 x 30 units
GAPDH Primer - Forward (20 µM)	1 x 5µl	1 x 10µl
GAPDH Primer - Reverse (20 µM)	1 x 5µl	1 x 10µl

Components	12 tests	24 tests
Lysis Buffer	1 x 7ml	1 x 14ml
MQ Binding Beads	1 x 1.6ml	2 x 1.6ml
Non-Immune IgG (1 mg/ml)	1 x 5µl	1 x 10µl
Primer I (10 µM)	1 x 15µl	1 x 30µl
Primer U (10 µM)	1 x 15µl	1 x 30µl
Proteinase K (10 mg/mL)	1 x 30µl	1 x 60µl
Rnase A	1 x 15µl	1 x 30µl
T4 DNA Ligase	1 x 15µl	1 x 30µl
Wash Buffer	1 x 12ml	1 x 25ml

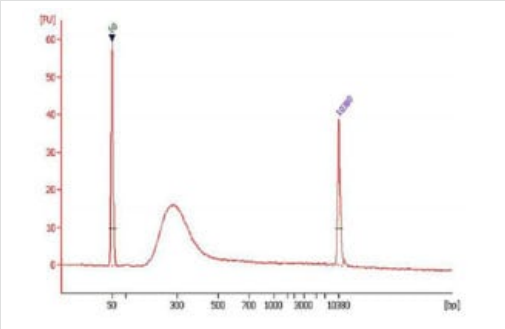
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab185908 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

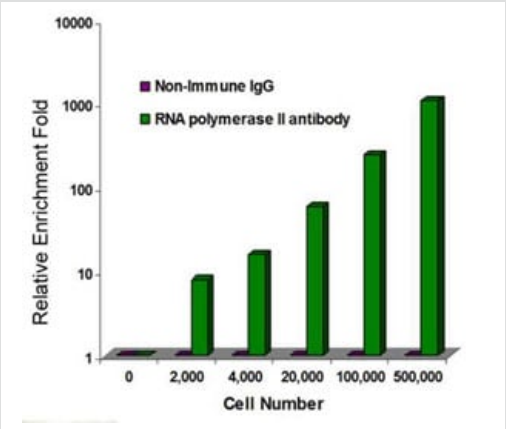
Application	Abreviews	Notes
ChIP-sequencing		Use at an assay dependent concentration.

Images



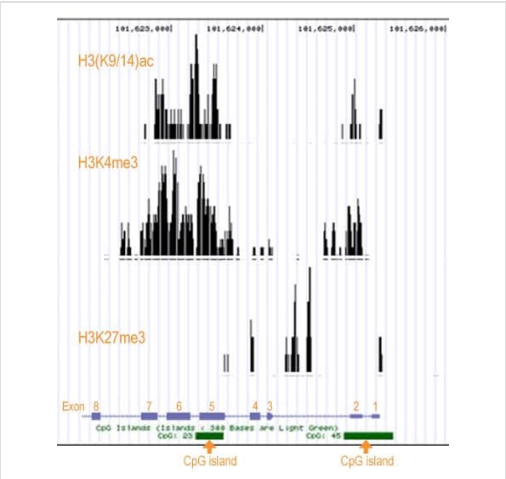
Size distribution of library fragments.

Ten nanograms of DNA was ChIPed by RNA polymerase II enrichment and used for DNA library preparation.



High sensitive ChIP

The sheared chromatin isolated from different number of MBD-231 cells was used for ChIP-qPCR analysis of RNA polymerase II enrichment in GAPDH promoters.



Sample results

A ChIP-sequencing library was prepared using ChIP-Seq High Sensitivity Kit (ab185908) from rat heart chromatin and polyclonal antibodies against H3K4me3, H3(K9/14)ac and, H3K27me3. Sequencing was carried out on an Illumina HiSeq2500. Bioinformatics analysis of ChIP-seq is performed utilizing Bowtie and MACS.

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