

Chromatin Accessibility Assay Kit ab185901

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Overview

Product name	Chromatin Accessibility Assay Kit
Sample type	Tissue, Adherent cells, Suspension cells
Assay type	Quantitative
Assay time	1h 30m
Species reactivity	Reacts with: Mammals
Product overview	Chromatin Accessibility Assay Kit ab185901 is a complete set of optimized reagents designed for conducting a gene-specific analysis of chromatin accessibility including nucleosome/transcription factor positioning from various biological samples via real time PCR.

The chromatin accessibility assay works on the principle that accessible chromatin is easily accessed by a nuclease mix to digest the DNA. When the DNA from the nuclease-treated sample and a no-nuclease control is analyzed for a particular gene by PCR, the more accessible the chromatin at that gene, the lower the signal of the nuclease-treated sample relative to the no-nuclease control.

Chromatin accessibility assay protocol summary:

- lyse cells and extract chromatin by adding lysis buffer to cell pellet, resuspending and incubating for 10 min
- centrifuge at 5,000 rpm for 5 min, discard supernatant, resuspend chromatin pellet in wash buffer and centrifuge again
- resuspend chromatin pellet in nuclease reaction mix (or no-nuclease control mix to controls) and incubate for 4 min
- add stop solution and incubate for 10 min
- add Proteinase K and incubate for 60 min
- add samples to DNA binding columns, spin, and discard flow through
- add DNA washing solution to columns, spin, and discard flow through 3 times
- add elution solution and spin to elute DNA
- analyze gene target with PCR and compare nuclease-treated to no-nuclease control

Notes The accessibility of regulatory elements in chromatin is critical for many aspects of gene regulation. Nucleosomes positioned over regulatory elements inhibit access of transcription factors to DNA. To elucidate the role of the interactions between chromatin and transcription factors, it is crucial to determine chromatin accessibility through mapping of the nucleosome positioning along the genome. In general, the more condensed the chromatin, the more difficult it is for transcription factors and other DNA binding proteins to access DNA and carry out their

tasks. The more accessible the DNA, the more likely surrounding genes are actively transcribed. The presence (or the absence) of nucleosomes directly or indirectly affects a variety of other cellular and metabolic processes such as recombination, replication, centromere formation, and DNA repair.

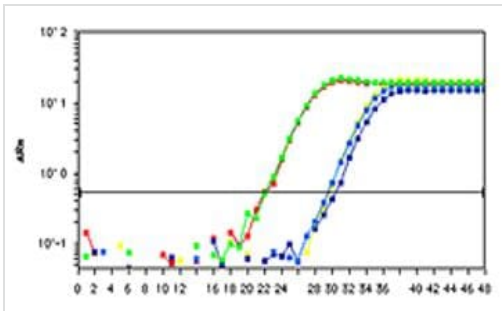
Properties

Storage instructions

Please refer to protocols.

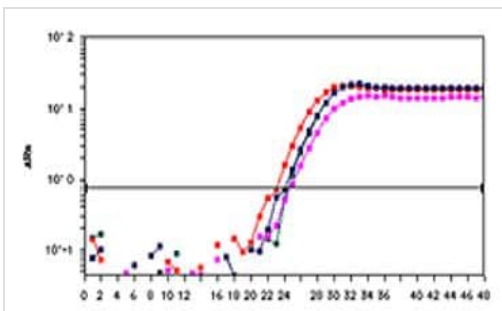
Components	48 tests
1000X Protease Inhibitor Cocktail	1 x 50µl
10X DNA Washing Solution	1 x 3ml
10X Lysis Buffer	1 x 11ml
10X Wash Buffer	1 x 10ml
DNA Binding Solution	1 x 15ml
Elution Solution	1 x 1ml
F-Collection Tube	1 x 50 units
F-Spin Column	1 x 50 units
Negative Control Primer-F (20 µM)	1 x 10µl
Negative Control Primer-R (20 µM)	1 x 10µl
Nuclear Digestion Buffer	1 x 3ml
Nuclease Mix	1 x 50µl
Positive Control Primer-F (20 µM)	1 x 10µl
Positive Control Primer-R (20 µM)	1 x 10µl
Proteinase K (10 mg/mL)	1 x 110µl
Reaction Stop Solution	1 x 0.5ml

Images



Chromatin accessibility of opened chromatin (euchromatin)

Amplification of proximal promoter regions for the constitutively expressed target gene was carried out in MDA-231 cells by using positive control primers. Red and green lines: Nse-untreated; Blue lines: Nse-treated. The difference of Ct values between Nse-treated and untreated is > 7 cycles.



Chromatin accessibility of closed chromatin (heterochromatin)

Amplification of proximal promoter regions for the constitutively repressed target gene was carried out in MDA-231 cells by using negative control primers. Red and green lines: Nse-untreated; Blue and pink lines: Nse-treated. The difference of Ct values between Nse-treated and untreated is <1.5 cycles.

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