abcam

Product datasheet

Anti-Cdk9 antibody [EPR22956-37] - ChIP Grade - BSA and Azide free ab259268

יילעבער RabMAb

画像数 14

製品の概要

Anti-Cdk9 antibody [EPR22956-37] - ChIP Grade - BSA and Azide free

製品の詳細 Rabbit monoclonal [EPR22956-37] to Cdk9 - ChIP Grade - BSA and Azide free

Rabbit

適用あり: Flow Cyt (Intra), ChIC/CUT&RUN-seq, IP, WB, IHC-P, ICC/IF, ChIP, ChIP-sequencing

種交差性 交差種: Mouse, Rat, Human

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

WB: HEK-293T, HeLa, RAW 264.7, PC-12, NIH/3T3 and C6 whole cell lysate; Mouse brain and rat lung tissue lysate; IHC-P: Human pancreas, pancreatic cancer, mouse and rat liver tissue. ICC/IF: HeLa and NIH/3T3 cells. Flow: HeLa and NIH/3T3 cells. IP: NIH/3T3 cell lysate. ChIP:

Chromatin from MEF and HeLa cells. ChlC/CUT&RUN-Seg: HeLa cells.

特記事項 ab259268 is the carrier-free version of ab239364.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

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製品名

由来種

アプリケーション

免疫原

ポジティブ・コントロール

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C. Do Not Freeze.

バッファー pH: 7.2

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 EPR22956-37

アイソタイプ lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab259268の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 42, 55 kDa (predicted molecular weight: 43 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
ChIP		Use 5 µg for 25 µg of chromatin.
ChIP-sequencing		Use 8µg for 10 ⁷ cells.

ターゲット情報

機能

Member of the cyclin-dependent kinase pair (CDK9/cyclin-T) complex, also called positive transcription elongation factor b (P-TEFb), which facilitates the transition from abortive to production elongation by phosphorylating the CTD (C-terminal domain) of the large subunit of RNA polymerase II (RNAP II), SUPT5H and RDBP. The CDK9/cyclin-K complex has also a

kinase activity toward CTD of RNAP II and can substitute for P-TEFb in vitro.

組織特異性 Ubiquito

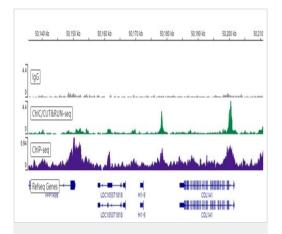
配列類似性Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX

subfamily.

Contains 1 protein kinase domain.

細胞内局在 Nucleus.

画像



ChIC/CUT&RUN sequencing - Anti-Cdk9 antibody
[EPR22956-37] - ChIP Grade - BSA and Azide free
(ab259268)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 5 μ g of <u>ab239364</u> [EPR22956-37]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative lgG control <u>ab172730</u> is also shown.

The ChIP data was conducted on chromatin prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HeLa cells and 8 μ g of <u>ab239364</u>. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

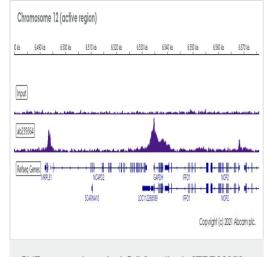
Additional screenshots of mapped reads can be downloaded here.

The University of Geneva owns patents relevant to ChlC (Chromatin Immuno-Cleavage) methods.

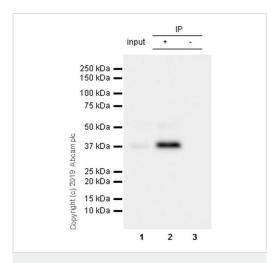
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab239364).

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HeLa cells and 8 μ g of <u>ab239364</u> [EPR22956-37]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

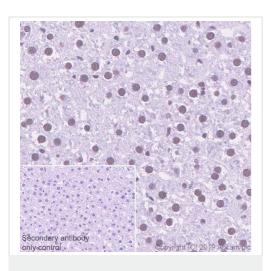
Additional screenshots of mapped reads can be downloaded $\underline{\textbf{here}}.$



ChIP-sequencing - Anti-Cdk9 antibody [EPR22956-37] - ChIP Grade - BSA and Azide free (ab259268)



Immunoprecipitation - Anti-Cdk9 antibody
[EPR22956-37] - ChIP Grade - BSA and Azide free
(ab259268)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cdk9 antibody

[EPR22956-37] - ChIP Grade - BSA and Azide free (ab259268)

CDK9 was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast), whole cell lysate 10 ug with <u>ab239364</u> at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab239364</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) was used at 1/5000 dilution.

Lane 1: NIH/3T3 (mouse embryonic fibroblast), whole cell lysate 10 ug

Lane 2: ab239364 IP in NIH/3T3 whole cell lysate

Lane 3: Rabbit monoclonal lgG ($\underline{ab172730}$) instead of $\underline{ab239364}$ in NIH/3T3 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 6 seconds

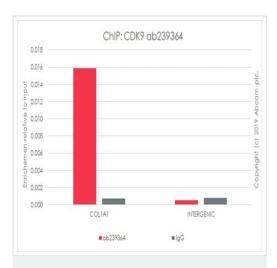
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab239364).

Immunohistochemical analysis of paraffin-embedded Rat liver tissue labeling CDK9 with <u>ab239364</u> at 1/2000 dilution (0.30 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Nuclear staining on rat liver (PMID: 9766517, 11282025). The section was incubated with <u>ab239364</u> for 10 mins at RT. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counterstained with Hematoxylin.

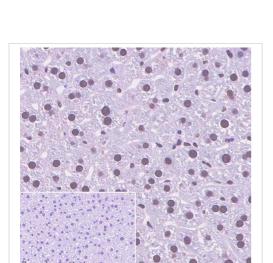
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab239364</u>).



ChIP - Anti-Cdk9 antibody [EPR22956-37] - ChIP Grade - BSA and Azide free (ab259268)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cdk9 antibody

[EPR22956-37] - ChIP Grade - BSA and Azide free (ab259268)

This data was developed using the same antibody clone in a different buffer formulation (<u>ab239364</u>).

Chromatin was prepared from MEF cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min. The ChIP was performed with 25 µg of chromatin, 5 µg of ab239364 (red), or 5 µg of rabbit normal IgG ab172730 (gray) and 20 µl of Protein A/G Sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci).

Primers and probes are from paper PMC4103662 (PMID: 23663783)

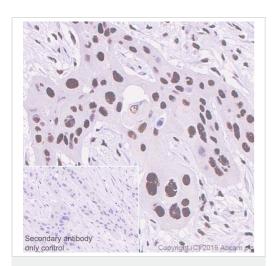
*http://www.abcam.com/resources? keywords=X%20ChIP%20protocol

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling CDK9 with ab239364 at 1/2000 dilution (0.30 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on mouse liver (PMID: 9766517, 11282025) The section was incubated with ab239364 for 10 mins at RT. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab239364).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cdk9 antibody

[EPR22956-37] - ChIP Grade - BSA and Azide free (ab259268)

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Cdk9 antibody

[EPR22956-37] - ChIP Grade - BSA and Azide free (ab259268)

Immunohistochemical analysis of paraffin-embedded Human pancreatic cancer tissue labeling CDK9 with <u>ab239364</u> at 1/2000 dilution (0.30 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Nuclear staining on human pancreatic cancer (PMID: 28231737) The section was incubated with <u>ab239364</u> for 10 mins at RT. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

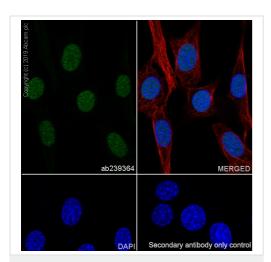
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab239364).

Immunohistochemical analysis of paraffin-embedded Human pancreas tissue labeling CDK9 with <u>ab239364</u> at 1/2000 dilution (0.30 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Nuclear staining on human pancreas (PMID: 28231737) The section was incubated with <u>ab239364</u> for 10 mins at RT. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab239364</u>).



Immunocytochemistry/ Immunofluorescence - Anti-Cdk9 antibody [EPR22956-37] - ChIP Grade - BSA and Azide free (ab259268)

ab239364 MERGED

DAPI Secondary antibody only control

Immunocytochemistry/ Immunofluorescence - Anti-Cdk9 antibody [EPR22956-37] - ChIP Grade - BSA and Azide free (ab259268)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labelling CDK9 with ab239364 at 1/100 (6 ug/ml) dilution, followed by ab150077 AlexaFluor[®] 488 Goat anti-Rabbit secondary antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing nuclear staining in NIH/3T3 cell line is observed. ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

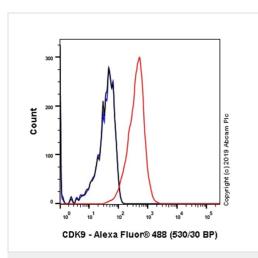
Secondary antibody only control: Secondary antibody is <u>ab239364</u> anti-CDK9 <u>ab150077</u> AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 (2 ug/ml) dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab239364).

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling CDK9 with <u>ab239364</u> at 1/100 (6 ug/ml) dilution, followed by <u>ab150077</u> AlexaFluor[®]488 Goat anti-Rabbit secondary antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing nuclear staining in HeLa cell line is observed. <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab239364</u> anti-CDK9 <u>ab150077</u> AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 (2 ug/ml) dilution.

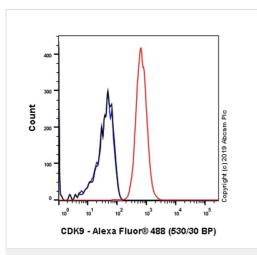
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab239364</u>).



Flow Cytometry (Intracellular) - Anti-Cdk9 antibody [EPR22956-37] - ChIP Grade - BSA and Azide free (ab259268)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (Mouse embryo) cells labelling CDK9 with ab239364 at 1/600 (Red) compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti-rabbit IgG (Alexa Fluor ab150077) at 1/2000 dilution was used as the secondary antibody.

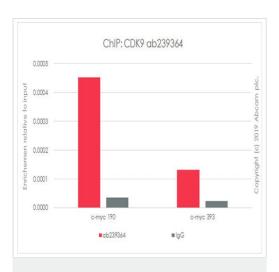
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab239364).



Flow Cytometry (Intracellular) - Anti-Cdk9 antibody [EPR22956-37] - ChIP Grade - BSA and Azide free (ab259268)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (Human cervix adenocarcinoma) cells labelling CDK9 with <u>ab239364</u> at 1/600 (Red) compared with a Rabbit monoclonal IgG (<u>ab172730</u>) / Black isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor[®]488, <u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab239364).



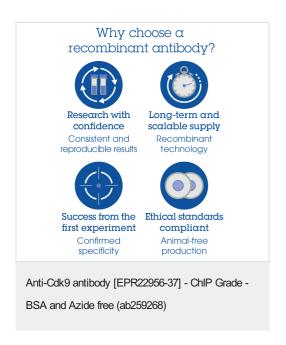
ChIP - Anti-Cdk9 antibody [EPR22956-37] - ChIP Grade - BSA and Azide free (ab259268)

Chromatin was prepared from HeLa cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.

The ChIP was performed with 25 μ g of chromatin, 5 μ g of ab239364 (red), or 5 μ g of rabbit normal lgG ab172730 (gray) and 20 μ l of Protein A/G Sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are from paper PMCID: PMC2756882.

*https://www.abcam.com/resources? keywords=X%20ChIP%20protocol

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab239364).



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