abcam

Product datasheet

Human H2AFY (mH2A1) knockout HEK-293T cell lysate ab257463

画像数 2

製品の概要

製品名	Human H2AFY (mH2A1) knockout HEK-293T cell lysate
製品の概要	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 13 bp deletion in exon 2.
Passage number	<20
Knockout validation	Sanger Sequencing
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
特記事項	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.
アプリケーション	**適用あり:** WB

製品の特性

保存方法 Store at -80°C. Please refer to protocols.

内容	1 kit
ab260244 - Human H2AFY knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

Cell type epithelial

STR Analysis Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12

ターゲット情報

機能 Variant histone H2A which replaces conventional H2A in a subset of nucleosomes where it represses transcription. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling. Involved in stable X chromosome inactivation. Inhibits the binding of transcription factors and interferes with the activity of remodeling SWI/SNF complexes. Inhibits histone acetylation by EP300 and recruits class I HDACs, which induces an hypoacetylated state of chromatin. In addition, isoform 1, but not isoform 2, binds ADP-ribose and O-acetyl-ADP-ribose, and may be involved in ADP-ribose-mediated chromatin modulation.

組織特異性 Ubiquitous.

配列類似性 Contains 1 histone H2A domain.
Contains 1 Macro domain.

翻訳後修飾 Monoubiquitinated at either Lys-116 or Lys-117. May also be polyubiquitinated. Ubiquitination is mediated by the CUL3/SPOP E3 complex and does not promote proteasomal degradation. Instead, it is required for enrichment in inactive X chromosome chromatin.

細胞内局在 Nucleus. Chromosome. Enriched in inactive X chromosome chromatin and in senescence-associated heterochromatin.

アプリケーション

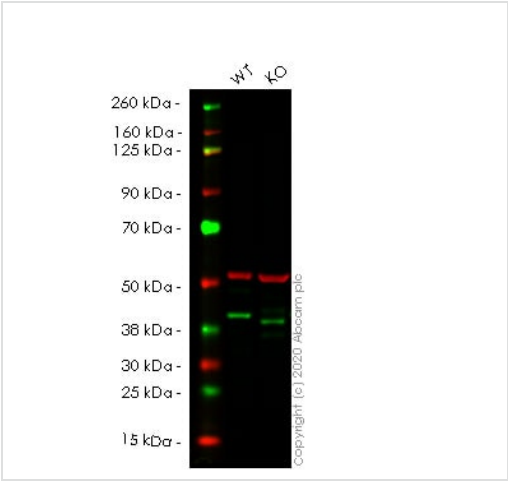
The Abpromise guarantee Abpromise保証は、 次のテスト済みアプリケーションにおけるab257463の使用に適用されます

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

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 40 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

画像



Western blot - Human H2AFY (mH2A1) knockout HEK293T cell lysate (ab257463)

Lane 1: Wild-type HEK-293T cell lysate (20µg)

Lane 2: H2AFY knockout HEK-293T cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab183041** observed at 40 kDa. Red - loading control, **ab7291** observed at 50 kDa.

ab183041 Anti-mH2A1 antibody [EPR9359(2)] was shown to specifically react with mH2A1 in wild-type HEK-293T cells in western blot. The band observed in the knockout cell line **ab266241** (knockout cell lysate ab257463) lane below 40kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and mH2A1 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab183041** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C at 1 in 1000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human H2AFY knockout HEK293T cell lysate (ab257463)

Homozygous: 13 bp deletion in exon 2

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