abcam

# Product datasheet

# Anti-CENPA antibody [3-19] - ChIP Grade ab13939

11 Abreviews 80 References 7 图像

## 概述

| | |
|---|---|
| **产品名称** | Anti-CENPA抗体[3-19] - ChIP Grade |
| **描述** | 小鼠单克隆抗体[3-19] to CENPA - ChIP Grade |
| **宿主** | Mouse |
| **经测试应用** | **适用于:** ICC/IF, IHC-P, WB, ChIP, Flow Cyt |
| **种属反应性** | **与反应:** Human<br>**不与反应:** Mouse, Rat |
| **免疫原** | Synthetic peptide:<br>PRRRSRKPEAPRRRSPS<br>, corresponding to amino acids 3-19 of Human CENP A.<br>Run BLAST with Run BLAST with |
| **阳性对照** | WB: Friend cell lysate ICC: human metaphase chromosomes |
| **常规说明** | For maximum product recovery centrifuge the product vial before removing cap. |

## 性能

| | |
|---|---|
| **形式** | Liquid |
| **存放说明** | Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle. |
| **存储溶液** | Preservative: None<br>Constituents: 50% Glycerol, PBS, pH 7.2 |
| **纯度** | Protein A purified |
| **纯化说明** | This antibody was purified using Protein A affinity chromatography. |
| **克隆** | 单克隆 |
| **克隆编号** | 3-19 |
| **同种型** | IgG1 |

## 应用

Our Abpromise guarantee covers the use of **ab13939** in the following tested applications.

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

| 应用 | Ab评论 | 说明 |
|---|---|---|
| ICC/IF | ★★★★★ | Use a concentration of 2 - 10 µg/ml. |
| IHC-P | | Use at an assay dependent concentration. |
| WB | ★★★★★ | Use a concentration of 1 µg/ml. Detects a band of approximately 18 kDa (predicted molecular weight: 16 kDa). |
| ChIP | | Use 4-25 µg for µg of chromatin. |
| Flow Cyt | | Use 1µg for $10^6$ cells.<br>ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody. |

## 靶标

**功能**

Histone H3-like variant which exclusively replaces conventional H3 in the nucleosome core of centromeric chromatin at the inner plate of the kinetochore. Required for recruitment and assembly of kinetochore proteins, mitotic progression and chromosome segregation. May serve as an epigenetic mark that propagates centromere identity through replication and cell division.

**序列相似性**

Belongs to the histone H3 family.

**结构域**

The CATD (CENPA targeting domain) region is responsible for the more compact structure of nucleosomes containing CENPA and is necessary and sufficient to mediate the localization into centromeres.

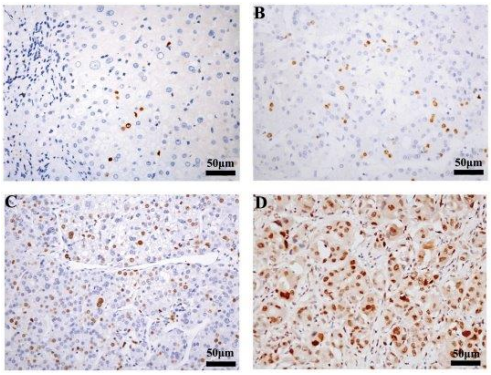
**翻译后修饰**

Ubiquitinated (Probable). Interaction with herpes virus HSV-1 ICP0 protein, leads to its degradation by the proteasome pathway.
Phosphorylation of Ser-7 by Aurora-A/STK6 and Aurora-B/STK12 during prophase is required for localization of Aurora-A/STK6 and Aurora-B/STK12 at inner centromere and is essential for kinetochore function. Initial phosphorylation during prophase is mediated by Aurora-A/STK6 and is maintained by Aurora-B/STK12.

**细胞定位**

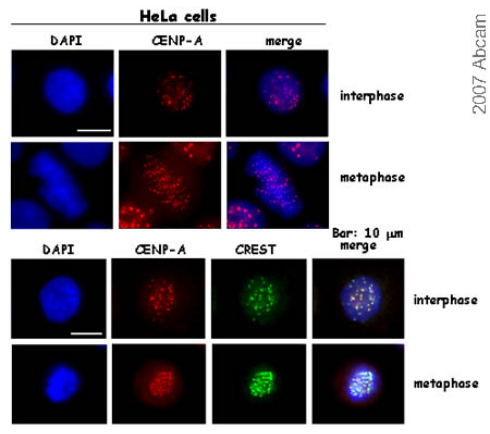
Nucleus. Chromosome > centromere > kinetochore. Localizes exclusively in the kinetochore domain of centromeres. Occupies a compact domain at the inner kinetochore plate stretching across 2 thirds of the length of the constriction but encompassing only one third of the constriction width and height.

## 图片




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CENPA antibody [3-19] - ChIP Grade (ab13939)

ab13939 staining CENPA in (A) nontumorous liver tissue, and in (B) well-differentiated, (C) moderately-differentiated, and (D) poorly-differentiated HCC. The CENP-A staining is predominantly nuclear in the samples examined, with concurrent diffuse cytoplasmic staining in poorly-differentiated HCC. Primary antibody used at a dilution of 1:500.

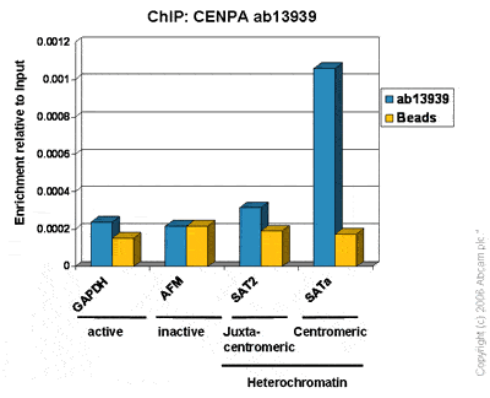



Immunocytochemistry/ Immunofluorescence - Anti-CENPA antibody [3-19] - ChIP Grade (ab13939)

Image and experimental conditions kindly provided by Serena Orlando, Giulia Guarguaglini and Patrizia Lavia, of the University 'La Sapienza' CNR, Italy

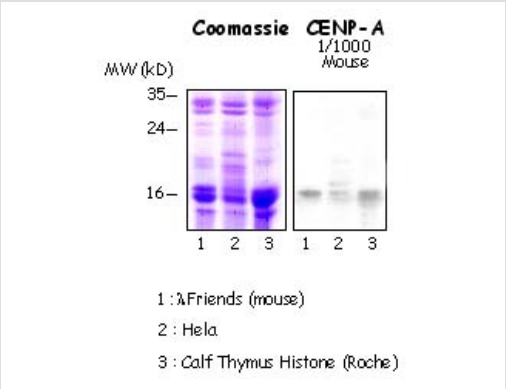
ab13939 staining CENPA by Immunofluorescence. This antibody works very well in HeLa cells, with double staining of kinetochores using CREST indicating perfect co-localization.

Optimal antibody dilution: 2mg/ml.




ChIP - Anti-CENPA antibody [3-19] - ChIP Grade (ab13939)

Chromatin was prepared from Hela cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 4µg of ab13939 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). 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 located in the first kb of the transcribed region.




Western blot - Anti-CENPA antibody [3-19] - ChIP Grade (ab13939)

This image is courtesy of Jerome Govin (Grenoble)

The antibody works well with histones from: Hela cells (human), Friend cells (mouse) and calf thymus (Roche). 5 µg acid-extracted histones were loaded per lane. Images taken following 5 min exposure. 1:1000 dilution.

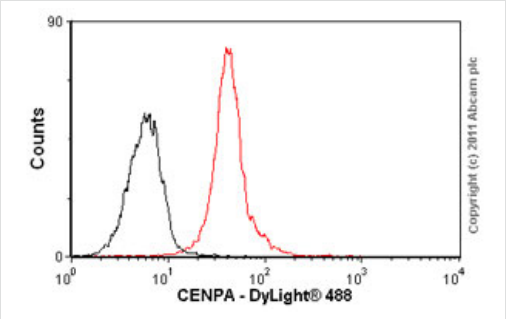
Lane 1: Friend cells (mouse)
Lane 2: Hela cells (human)
Lane 3: Calf thymus

The antibody works well with histones from: Hela cells (human), Friend cells (mouse) and calf thymus (Roche). 5 µg acid-extracted histones were loaded per lane. Images taken following 5 min exposure. 1:1000 dilution.

Lane 1: Friend cells (mouse)

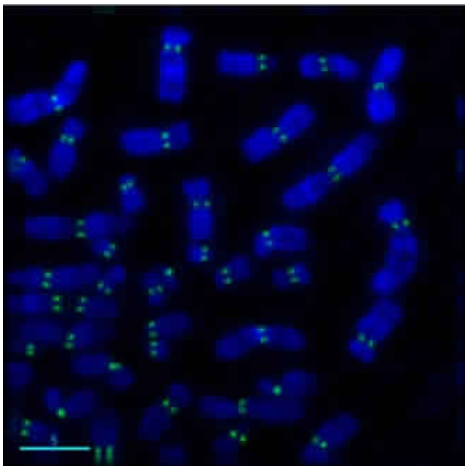
Lane 2: Hela cells (human)

Lane 3: Calf thymus



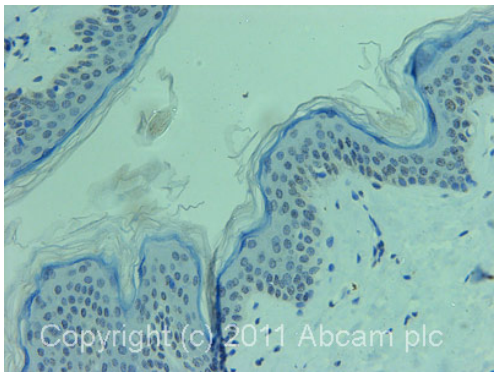

Flow Cytometry - Anti-CENPA antibody [3-19] - ChIP Grade (ab13939)

Overlay histogram showing HeLA cells stained with ab13939 (red line). The cells were fixed with 100% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab13939, 1µg/1x$10^6$ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was Mouse IgG1 [ICIGG1] (ab91353, 2µg/1x$10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed.



Immunocytochemistry/ Immunofluorescence - Anti-CENPA antibody [3-19] - ChIP Grade (ab13939)

This picture shows human metaphase chromosomes detected with ab13939 as primary antibody and AF488 goat anti-mouse secondary antibody (green). This image was kindly supplied as part of the review submitted by Professor Beth A. Sullivan, Boston University School of Medicine.




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CENPA antibody [3-19] - ChIP Grade (ab13939)

IHC image of ab13939 staining in normal human skin formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab13939, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



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