# abcam

## Product datasheet

## Anti-CLLD8/SETDB2 antibody ab5517

4 References 5 图像

概述

产品名称 Anti-CLLD8/SETDB2抗体

描述 兔多克隆抗体to CLLD8/SETDB2

**宿主** Rabbit

经测试应用 适用于: WB, ICC/IF

种属反应性 与反应: Human, Fall armyworm

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

常规说明 Our collaborator on this project has found that the antibody works poorly in WB.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

性能

形式 Liquid

**存放说明** Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw

cycles.

**存储溶液** pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

纯**度** Immunogen affinity purified

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#### The Abpromise guarantee

## Abpromise™承诺保证使用ab5517于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 82 kDa.
ICC/IF		Use at an assay dependent concentration.

#### 靶标

#### 功能

Histone methyltransferase involved in left-right axis specification in early development and mitosis. Specifically trimethylates 'Lys-9' of histone H3 (H3K9me3). H3K9me3 is a specific tag for epigenetic transcriptional repression that recruits HP1 (CBX1, CBX3 and/or CBX5) proteins to methylated histones. Contributes to H3K9me3 in both the interspersed repetitive elements and centromere-associated repeats. Plays a role in chromosome condensation and segregation during mitosis.

#### 组织特异性

Ubiquitous. Highest expression in heart, testis and ovary.

#### 序列相似性

Belongs to the histone-lysine methyltransferase family. Contains 1 MBD (methyl-CpG-binding) domain.

Contains 1 pre-SET domain.

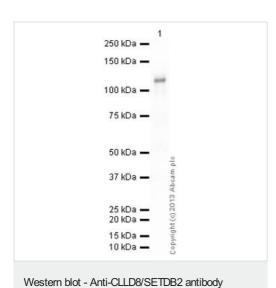
Contains 1 SET domain.

## 细胞定位

Nucleus. Chromosome.

#### 图片

(ab5517)



Anti-CLLD8/SETDB2 antibody (ab5517) at 1 µg/ml + Recombinant Human CLLD8/SETDB2 protein (ab169554) at 0.01 µg

## Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 82 kDa

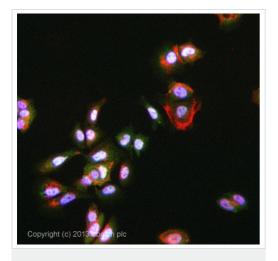
Additional bands at: 120 kDa (possible tagged protein)

Exposure time: 10 seconds

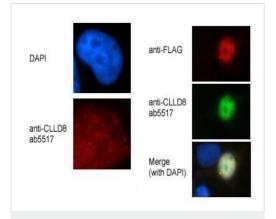
ab5517 recognises a band corresponding to the tagged CLLD8/SETB2 at approximately 120 kDa.

Abcam recommends using milk as the blocking agent. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above.

ICC/IF image of ab5517 stained MCF-7 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal Goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab5517 at 5ug/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 Goat anti Rabbit (ab96899) lgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43 $\mu$ M.



Immunocytochemistry/ Immunofluorescence - Anti-CLLD8/SETDB2 antibody (ab5517)



Immunocytochemistry/ Immunofluorescence - Anti-CLLD8/SETDB2 antibody (ab5517)

This image is courtesy of Genevieve Fourel, Grenoble

#### Left: Endogenous CLLD8

Detection using indirect fluorescence of the signal corresponding to endogenous CLLD8 in 293T cells. Cells fixed using 4% formaldehyde, blocked with PBS containing 3% milk and 0.5% Triton X-100, incubated for 1 hour at 37 °C using a 1/50 dilution of antibody ab5517. Cells were then washed 3 times and incubated for 1 hour at 37 °C with a goat anti-rabbit secondary antibody (1/500 dilution) coupled to Alexa Fluor 555 (Molecular Probes). Following 3 more washes, cells were stained with DAPI and mounted with Vectashield.

Top: DAPI

Bottom: ab5517

## Right: Overexpressed CLLD8

Detection using indirect fluorescence of the signal corresponding to staining with anti-FLAG mouse antibody (top) and an antibody (ab5517) against CLLD8 (middle) in 293T cells. 293T cells were transfected with vectors for overexpression of flagged CLLD8 using Lipofectamine 2000 transfection procedure (Invitrogen). Cells were fixed 48 hours post-tra

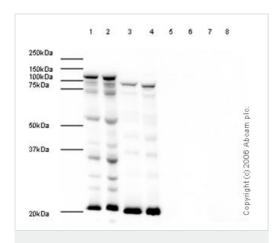


Immunocytochemistry/ Immunofluorescence - Anti-CLLD8/SETDB2 antibody (ab5517)

This image is courtesy of Genevieve Fourel, Grenoble

Detection using indirect fluorescence of the signal corresponding to endogenous CLLD8 in 293T cells. Using Lipofectamine 2000 (Invitrogen), cells were transfected with either a control shRNA directed against luciferase (left, SiRluc) or a specific siRNA directed against CLLD8 (right). 48 hours post-transfection, cells were fixed, blocked, and incubated with a 1:50 dilution of ab5517 at 37 °C for 1 hour. Cells were then washed 3 times and incubated at 37 °C for 1 hour with a 1/500 dilution of goat anti-rabbit antibody coupled with Alexa Fluor 555 (Molecular Probes). Following 3 further washes cells were stained with DAPI and mounted with Vectashield.

Antibody signals were extinguished when a specific RNAi was used. The few cells which retained a signal were presumably not transfected.



Western blot - Anti-CLLD8/SETDB2 antibody (ab5517)

All lanes: Anti-CLLD8/SETDB2 antibody (ab5517) at 1 µg/ml

Lane 1 : Sf21 cells infected by baculovirus containing FLAG-CLLD8 gene at 10 µg

**Lane 2**: Sf21 cells infected by baculovirus containing FLAG-CLLD8 gene at 20 µg

**Lane 3**: non-infected Sf21 cells at 10 μg **Lane 4**: non-infected Sf21 cells at 20 μg

**Lane 5 :** Sf21 infected by baculovirus containing FLAG-CLLD8 gene at 10  $\mu$ g with Human CLLD8/SETDB2 peptide (ab24398) at 1  $\mu$ g/ml

**Lane 6 :** Sf21 infected by baculovirus containing FLAG-CLLD8 gene at 20  $\mu$ g with Human CLLD8/SETDB2 peptide (<u>ab24398</u>) at 1  $\mu$ g/ml

**Lane 7**: non-infected Sf21 cells at 10  $\mu g$  with Human

CLLD8/SETDB2 peptide (ab24398) at 1 µg/ml

**Lane 8 :** non-infected Sf21 cells at 20  $\mu$ g with Human CLLD8/SETDB2 peptide (ab24398) at 1  $\mu$ g/ml

**Predicted band size:** 82 kDa **Observed band size:** 110 kDa

Additional bands at: 22 kDa (possible non-specific binding), 90

kDa (possible non-specific binding)

ab5517 recognises a band corresponding to FLAG-tagged CLLD8/SETB2 at approximately 110 kDa in Sf21 cells infected by baculovirus expressing CLLD8/SETDB2 (lanes1-2). This is larger than the predicated band size (81 kDa) due to the addition of the FLAG tag.

ab5517 does not detect a band in uninfected sf21 cells (lanes3-4) or following blocking studies using the immunizing peptide (lanes5-6).

There appears to be a ladder of proteins recognised by ab5517 (lanes1-2), which could be attributed to degradation products.

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