abcam

Product datasheet

Human Histone H3 (Mutated K27M, K36M) + Histone H3.3 (Mutated G34W, G34V, G34R) Antibody Panel ab274411

重组

32 图像

概述

产品名称 人Histone H3 (Mutated K27M, K36M) + Histone H3.3 (Mutated G34W, G34V, G34R)抗体组合

种属反应性 **与反应:** Human

产品概述 Human Histone H3 (Mutated K27M, K36M) + Histone H3.3 (Mutated G34W, G34V, G34R) Antibody Panel ab274411 contains multiple trial-sized versions of anti-human antibody clones against Histone H3 (Mutated K27M), Histone H3 (Mutated K36M), Histone H3.3 (Mutated G34W), Histone H3.3 (Mutated G34V) and Histone H3.3 (Mutated G34R), specifically selected for high performance in various applications. They are provided as a sampler panel to allow you to easily evaluate each antibody.

For guidelines on how to use each antibody within the panel, please consult the individual datasheet for each antibody.

Panel contains:

- Rabbit monoclonal [EPR18340] to H3K27M (20 µL) **ab190631**

- Rabbit monoclonal [EPR23614-91] to H3K36M (20 µL) **ab256384**

- Rabbit monoclonal [EPR23581-39] to H3G34W (20 µL) **ab272691**

- Rabbit monoclonal [EPR23520-5] to H3G34V (20 µL) **ab254401**

- Rabbit monoclonal [EPR23519-91] to H3G34R (20 µL) **ab254402**

说明 **Explore our range of antibody sample panels** designed to provide you with a variety of trial-size antibodies in a convenient and cost-effective format.

Directly conjugated versions of our antibodies are available and ready to use for multicolor flow cytometry or immunocytochemistry analysis. Please refer to the 'Associated products' section below.

Carrier-free formulations of our recombinant antibodies are also available for easy conjugation

to labels of your choice and for multiplex applications. Please refer to the ‘Associated products’ section below.

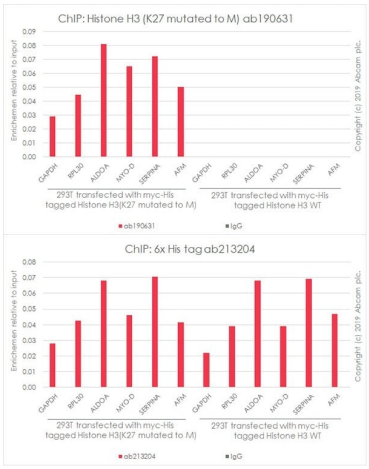
性能

存放说明 Store at -20°C. Please refer to protocols.

组件	1 kit
ab190631 - Anti-Histone H3 (mutated K27 M) antibody [EPR18340] - ChIP Grade	2 x 10µl
ab256384 - Anti-Histone H3 (mutated K36 M) antibody [EPR23614-91]	2 x 10µl
ab254402 - Anti-Histone H3.3 (mutated G34 R) antibody [EPR23519-91] -ChIP Grade	2 x 10µl
ab254401 - Anti-Histone H3.3 (mutated G34 V) antibody [EPR23520-5] - ChIP Grade	2 x 10µl
ab272691 - Anti-Histone H3.3 (mutated G34 W) antibody [EPR23581-39] - ChIP Grade	2 x 10µl

细胞定位 Histone H3.3: Nucleus. Chromosome. Histone H3: Nucleus. Chromosome.

图片

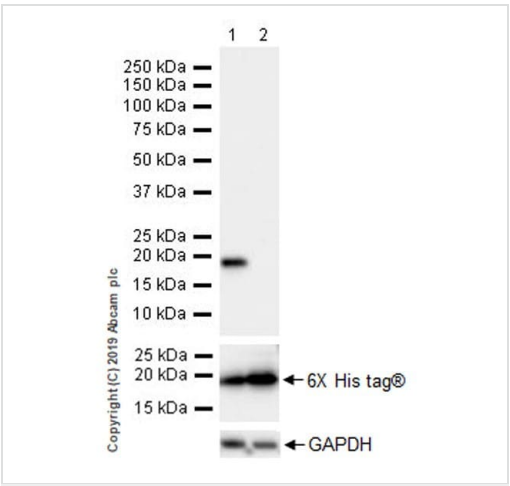


ChIP - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade

Chromatin was prepared from HEK-293T transfected with myc-His tagged Histone H3(K27 M) and Histone H3 WT 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, 2 µg of **ab190631** (red), 2 µg of **ab213204** (red) (bottom panel, served as internal control) or 2 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 located in the first kb of the transcribed region.

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



Western blot - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade

All lanes: Anti-Histone H3 (mutated K27 M) antibody [EPR18340] (**ab190631**) at 1/1000 dilution.

Lane 1: HEK-293 transfected with Histone H3.1 (K27M) expression vector containing a myc-His-tag, whole cell lysate, 10 ug.

Lane 2: HEK-293 transfected with Histone H3(WT) expression vector containing a myc-His-tag, whole cell lysate, 10 ug.

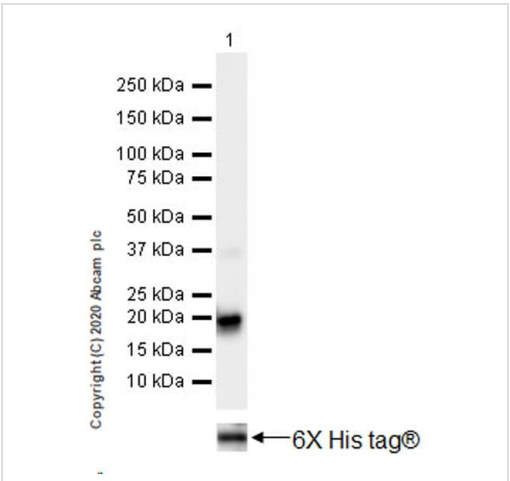
Secondary (all lanes): ab97051 at 1/100000 dilution.

Predicted MW: 15 kDa.

Observed MW: 18 kDa.

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

Exposure time: 6 seconds.



Western blot - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade

All lanes: Anti-Histone H3 (mutated K27 M) antibody [EPR18340] (**ab190631**) at 1/1000 dilution.

Lane 1: HEK-293 transfected with Histone H3.3 (K27M) expression vector containing a myc-His-tag®, whole cell lysate, 10 ug

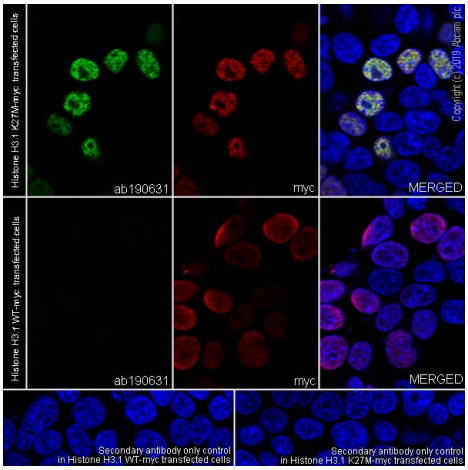
Secondary (all lanes): ab97051 at 1/100000 dilution.

Predicted MW: 15 kDa.

Observed MW: 18 kDa.

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

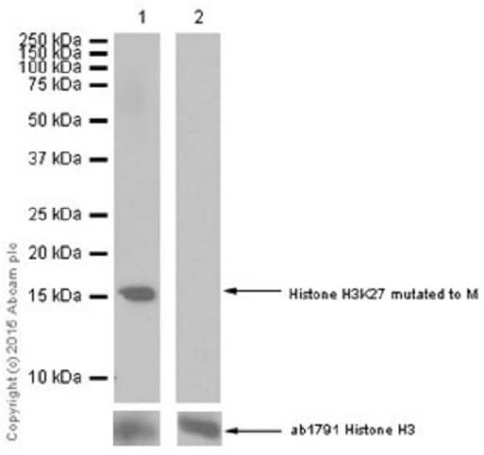
Exposure time: 3 seconds.



Immunocytochemistry/Immunofluorescence - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (human embryonic kidney epithelial cell) cells labelling Histone H3 (K27 mutated to M) with **ab190631** at 1/5000 (0.2 ug/ml) dilution, followed by **ab190631** anti- H3(K27 mutated to M) **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing nuclear staining in HEK-293T cells transfected with myc-tagged H3 (K27 mutated to M) expression vector 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 Ab190631 anti- H3(K27 mutated to M) **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 (2 ug/ml) dilution.



Western blot - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade

All lanes: Anti-Histone H3 (mutated K27 M) antibody [EPR18340] (**ab190631**) at 1/1000 dilution.

Lane 1: His-tagged recombinant histone H3 K27M protein, 0.01 ug

Lane 2: His-tagged recombinant wild type histone H3 protein, 0.01 ug

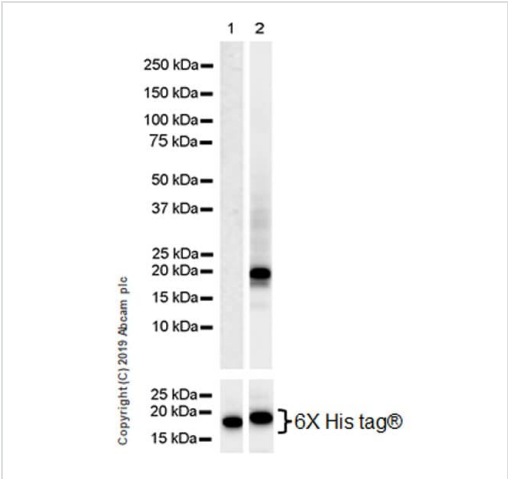
Secondary (all lanes): **ab97051** at 1/100000 dilution.

Predicted MW: 15 kDa.

Observed MW: 15 kDa.

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

Exposure time: 15 seconds.



Western blot - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91]

All lanes: Anti-Histone H3 (mutated K36 M) antibody [EPR23614-91] (**ab256384**) at 1/1000 dilution.

Lane 1: HEK-293 transfected with Histone H3.3 (WT) expression vector containing a myc-His-tag®, whole cell lysate, 10 ug

Lane 2: HEK-293 transfected with Histone H3.3 K36M (mutated) expression vector containing a myc-His-tag®, whole cell lysate, 10 ug.

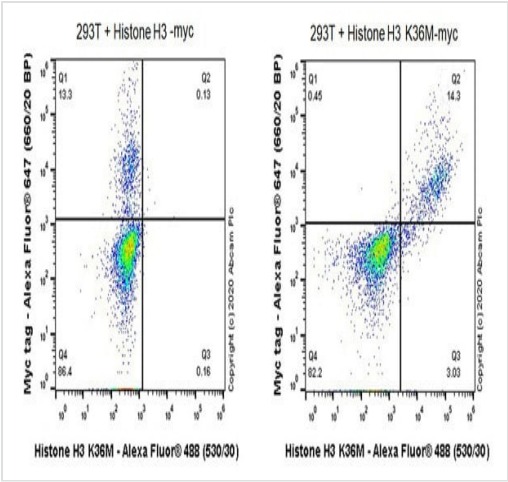
Secondary (all lanes): **ab97051** at 1/100000 dilution.

Predicted MW: 15 kDa.

Observed MW: 20 kDa.

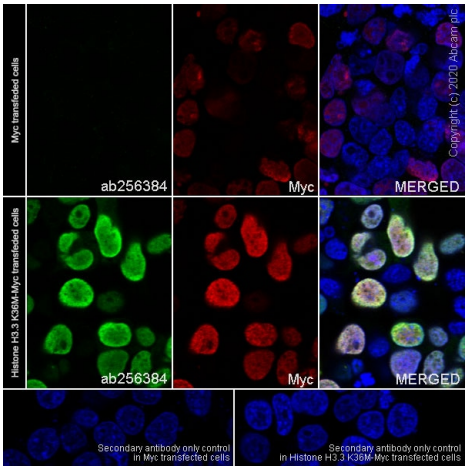
Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 26 seconds



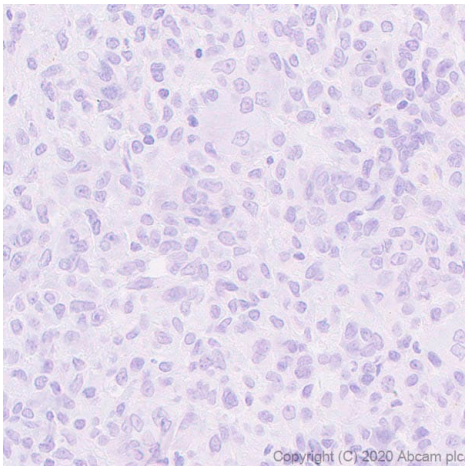
Flow Cytometry (Intracellular) - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91]

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HEK-293T transfected with myc tagged Histone H3 construct (Left) or myc tagged Histone H3 K36M construct (Right) cells labelling Histone H3 (mutated K36 M) with **ab256384** at 1/500 compared with a isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 was used as the secondary antibody.



Immunocytochemistry/Immunofluorescence - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91]

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HEK-293 cells labelling Histone H3 (mutated K36 M) with **ab256384** at 1/50 dilution, followed by a secondary antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in HEK-293 cell line transfected with myc-tagged Histone H3 K36M expression vector. 2233S Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 647 Conjugate) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

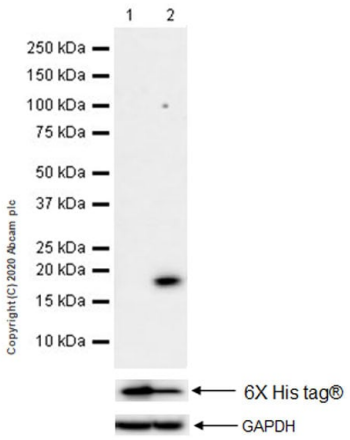


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91]

Immunohistochemical analysis of paraffin-embedded human giant cell tumor of bone tissue labeling Histone H3 (mutated K36 M) with **ab256384** at 1/4000 dilution followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). **Negative control:** No staining in human giant cell tumor of bone (PMID: 29757500). Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0)



Western blot - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91]

All lanes: Anti-Histone H3 (mutated K36 M) antibody [EPR23614-91] (**ab256384**) at 1/1000 dilution.

Lane 1: HEK-293 transfected with Histone H3.3 (WT) expression vector containing a myc-His-tag®, whole cell lysate, 10 ug

Lane 2: HEK-293 transfected with Histone H3.3 K36M (mutated) expression vector containing a myc-His-tag®, whole cell lysate, 10 ug.

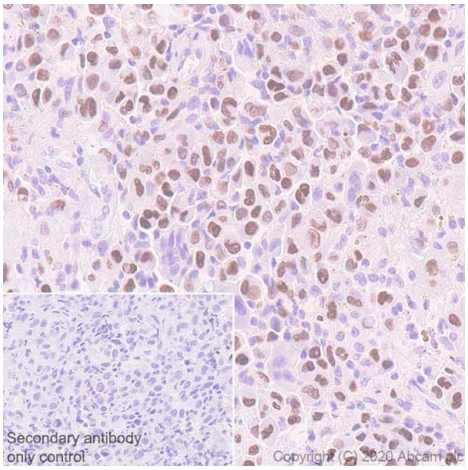
Secondary (all lanes): **ab97051** at 1/100000 dilution.

Predicted MW: 15 kDa.

Observed MW: 20 kDa.

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

Exposure time 62 seconds.

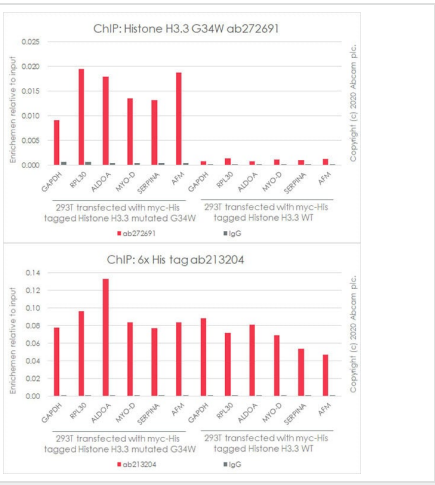


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91]

Immunohistochemical analysis of paraffin-embedded human chondroblastoma tissue labeling Histone H3 (mutated K36 M) with **ab256384** at 1/4000 dilution followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining in human chondroblastoma (PMID: 29757500). Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0)

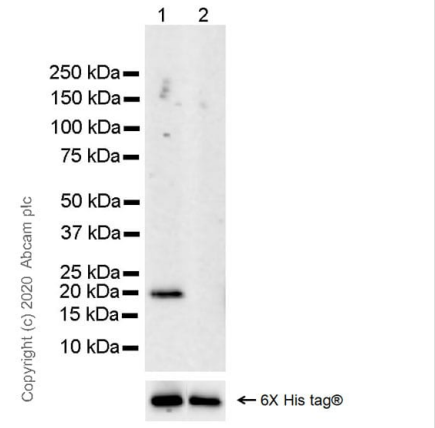


ChIP - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

Chromatin was prepared from HEK-293T transfected with myc-His tagged Histone H3.3 mutated G34W and Histone H3.3 WT cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 2 µg of **ab272691** (red), or 2 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 located in the first kb of the transcribed region.

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



Western blot - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

All lanes: Anti-Histone H3.3 (mutated G34 W) antibody [EPR23581-39] - ChIP Grade (**ab272691**) at 1/1000 dilution.

Lane 1: HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34W expression vector containing a myc-His-tag®, whole cell lysate, 40 ug.

Lane 2: HEK-293T transfected with Histone H3.3 (WT) expression vector containing a myc-His-tag®, whole cell lysate, 40 ug.

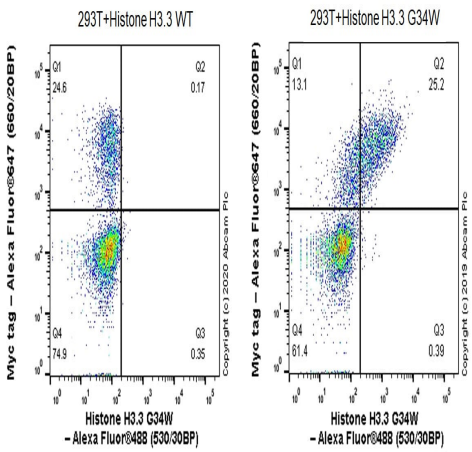
Secondary (all lanes): Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**), 1/50000 dilution.

Predicted MW: 15 kDa.

Observed MW: 20 kDa.

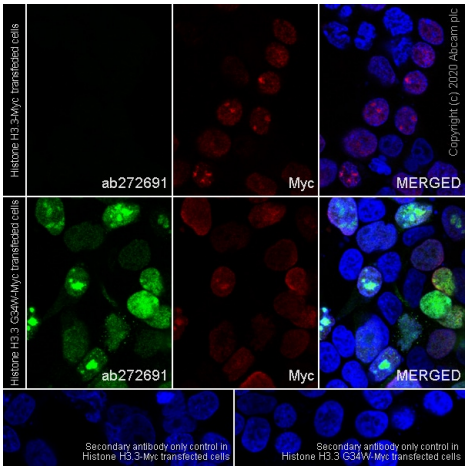
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.



Flow Cytometry (Intracellular) - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

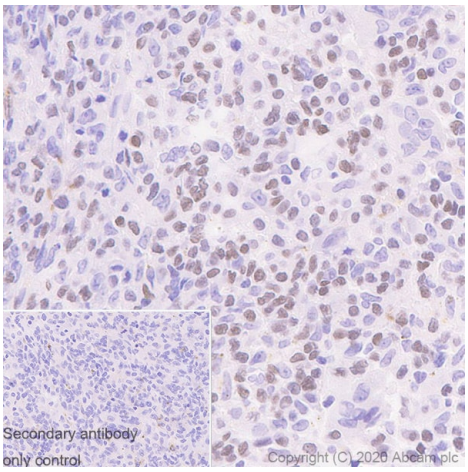
Flow cytometric analysis of 4% paraformaldehyde-fixed 90% methanol-permeabilized HEK-293T (Human embryonic kidney epithelial cell) transfected with myc tagged Histone H3.3 WT construct (Left panel) and myc-tagged Histone H3.3 G34W construct (Right panel) cells labelling Histone H3.3(mutated G34 W) with **ab272691** at 1/50 dilution (1µg). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/Immunofluorescence - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T cells labelling Histone H3.3(mutated G34 W) with **ab272691** at 1/100 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2 µg/ml dilution (Green). Confocal image showing nuclear staining in HEK-293T cells transfected with Histone H3.3 G34W-Myc plasmid, while no staining in HEK-293T cells transfected with H3.3 WT -Myc plasmid. Myc-Tag Mouse mAb (Alexa Fluor® 647) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 2 µg/ml dilution.

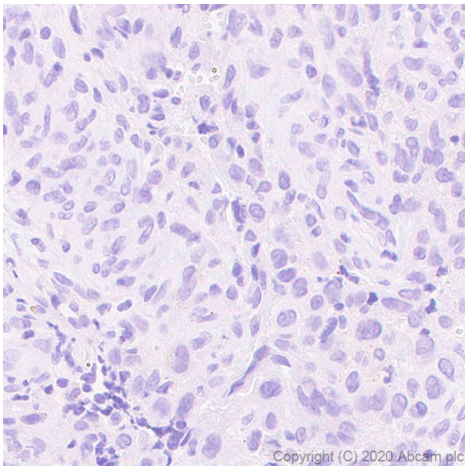


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

Immunohistochemical analysis of paraffin-embedded human giant cell tumor of bone tissue labeling Histone H3.3(mutated G34 W) with **ab272691** at 1/250 dilution followed by ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining in human giant cell tumor of bone (PMID: 29757500). Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

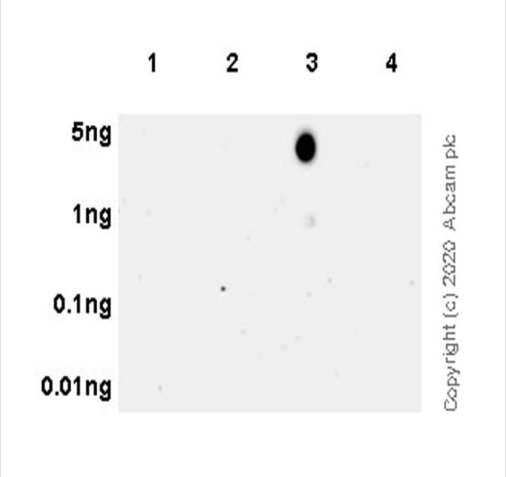
Immunohistochemical analysis of paraffin-embedded human chondroblastoma tissue labeling Histone H3.3(mutated G34 W) with **ab272691** at 1/250 dilution followed by ready to use Goat Anti-Rabbit IgG H&L (HRP).

Negative control: No staining in human chondroblastoma (PMID: 29757500).

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Dot Blot - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade

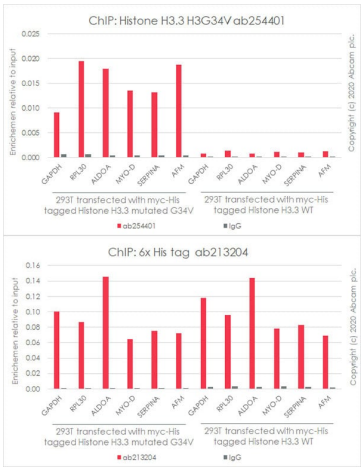
Dot blot analysis of Histone H3.3 (mutated G34 W) labeled with **ab272691** at 1/1000 dilution.

Lane 1: Histone H3.3 H3G34W peptide (aa28-37).
Lane 2: Histone H3.3 H3G34W peptide (aa33-43).
Lane 3: Histone H3.3 H3G34W peptide (aa28-43).
Lane 4: Histone H3.3 WT peptide (aa28-43).

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

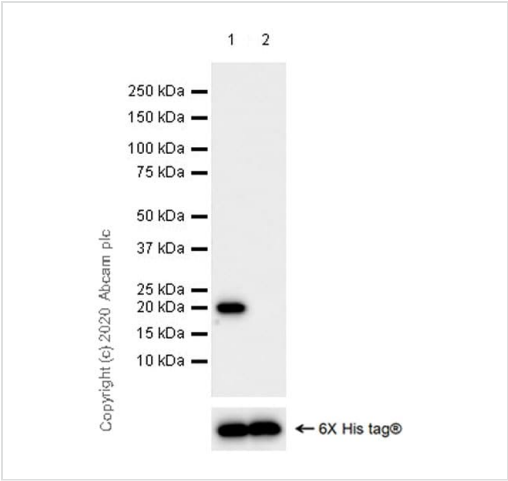
Exposure time: 3 minutes.



ChIP - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

Chromatin was prepared from HEK-293T transfected with myc-His tagged Histone H3.3 mutated G34V and Histone H3.3 WT cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with then formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 2 µg of **ab254401** (red), or 2 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 located in the first kb of the transcribed region.

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



Western blot - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

All lanes: Anti-Histone H3.3 (mutated G34 V) antibody [EPR23520-5] - ChIP Grade (**ab254401** at 1/1000 dilution.

Lane 1: HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34V expression vector containing a myc-His-tag®, whole cell lysate, 20 ug

Lane 2: HEK-293T transfected with Histone H3.3 (WT) expression vector containing a myc-His-tag®, whole cell lysate, 20 ug.

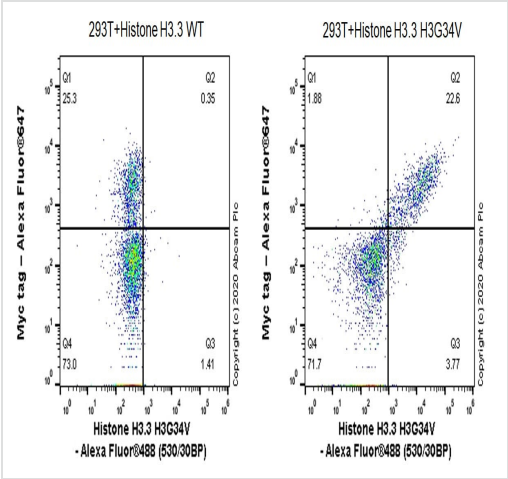
Secondary (all lanes): Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution.

Predicted MW: 15 kDa.

Observed MW: 20 kDa.

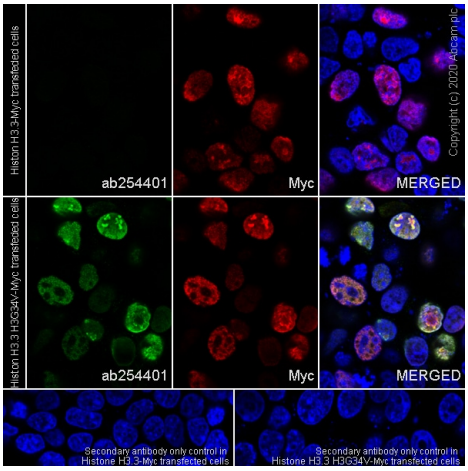
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.



Flow Cytometry (Intracellular) - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

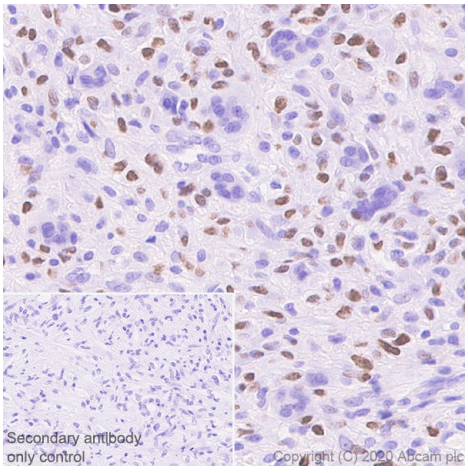
Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HEK-293T (Human embryonic kidney epithelial cell) transfected with myc tagged Histone H3.3 WT construct (Left panel) and myc-tagged Histone H3.3 H3G34V construct (Right panel) cells labelling Histone H3.3(mutated G34 V) with **ab254401** at 1/500 dilution (0.1µg). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/Immunofluorescence - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T cells labelling Histone H3.3(mutated G34 V) with **ab254401** at 1/1000 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2 µg/ml dilution (Green). Confocal image showing nuclear staining in HEK-293T cells transfected with Histone H3.3 H3G34V-Myc plasmid, while no staining in HEK-293T cells transfected with H3.3 WT -Myc plasmid. Myc-Tag Mouse mAb (Alexa Fluor® 647) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 2 µg/ml dilution.

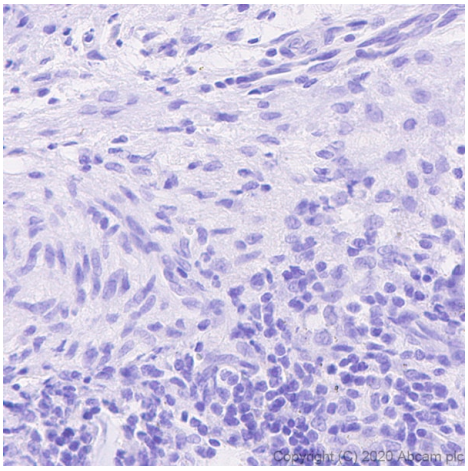


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

Immunohistochemical analysis of paraffin-embedded human giant cell tumor of bone tissue labeling Histone H3.3(mutated G34 V) with **ab254401** at 1/1000 dilution (0.542 µg/ml) dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human giant cell tumor of bone. (PMID: 29241742). The section was incubated with **ab254401** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is 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 solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

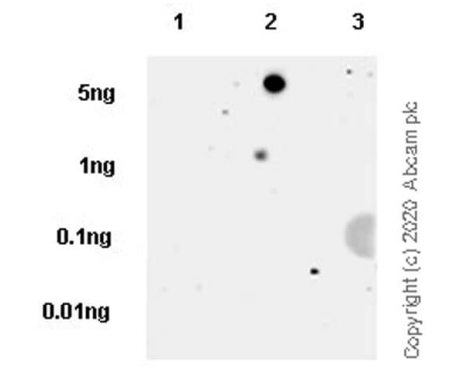
Immunohistochemical analysis of paraffin-embedded human chondroblastoma tissue labeling Histone H3.3(mutated G34 V) with **ab254401** at 1/1000 dilution (0.542 µg/ml) dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Negative control: No staining on human chondroblastoma (PMID: 29241742).

The section was incubated with **ab254401** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is 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 solution2) for 20 mins.



Dot Blot - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade

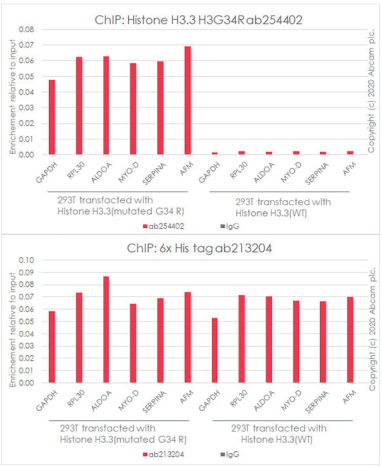
Dot blot analysis of Histone H3.3 (mutated G34 V) labeled with **ab254401** at 1/1000 dilution.

Lane 1: Histone H3.3 H3G34V peptide (aa28-40).
Lane 2: Histone H3.3 H3G34V peptide (aa26-38).
Lane 3: Histone H3.3 WT peptide (aa26-40).

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

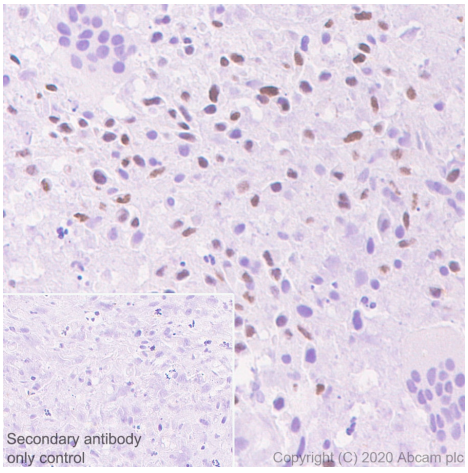
Exposure time: 3 minutes.



ChIP - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade

Chromatin was prepared from HEK-293T transfected with Histone H3.3(mutated G34 R) and HEK-293T transfacted with Histone H3.3(WT) cells according to the Abcam X-ChIP protocol*. Cells were fixed with formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 2 µg of **ab254402** (red), or 2 µg of rabbit normal IgG **ab172730** (gray) and 25 µl of Protein A/G Dynabeads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci).

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

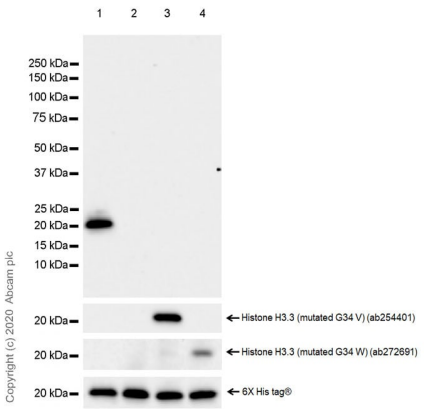


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade

Immunohistochemical analysis of paraffin-embedded human giant cell tumor of bone tissue labeling Histone H3.3 (mutated G34 R) with **ab254402** at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on human giant cell tumor of bone (PMID: 29241742). The section was incubated with **ab254402** for 30 mins at room temperature. 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 Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins



Western blot - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade

All lanes: Anti-Histone H3.3 (mutated G34 R) antibody [EPR23519-91] - ChIP Grade (**ab254402**) at 1/1000 dilution.

Lane 1: HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34R expression vector containi a myc-His-tag®, whole cell lysate, 20 ug.

Lane 2: HEK-293T transfected with Histone H3.3 (WT) expression vector containing a myc-His-tag®, whole cell lysate, 20 ug.

Lane 3: HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34V expression vector containing a myc-His-tag®, whole cell lysate, 20 ug.

Lane 4: HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34W expression vector containngi a myc-His-tag®,

whole cell lysate, 20 ug.

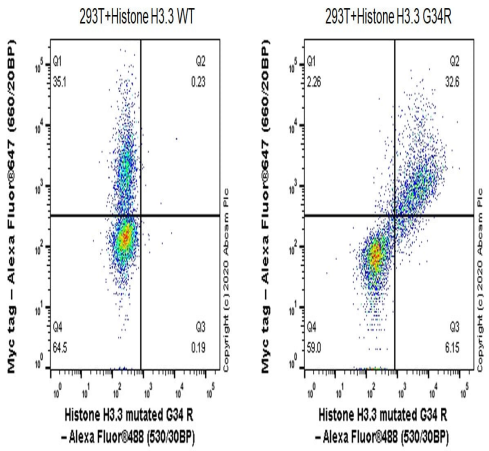
Secondary (all lanes): Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**), 1/100000 dilution.

Predicted MW: 15 kDa.

Observed MW: 20 kDa.

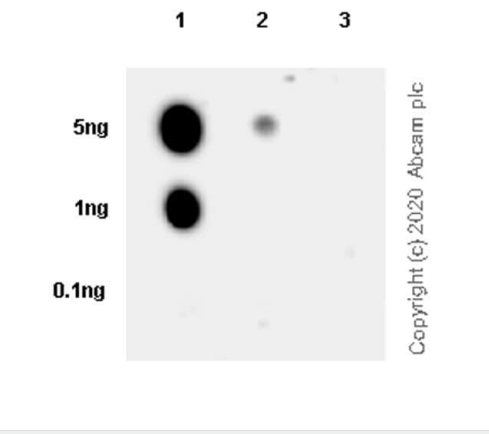
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Exposure time: 26 seconds.



Flow Cytometry (Intracellular) - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade

Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HEK-293T (Human embryonic kidney epithelial cell) (transfected with myc-tagged Histone H3.3 WT expression vector) (Left) or myc-tagged Histone H3.3 G34R expression vector (Right) cells labelling Histone H3.3 (mutated G34 R) with **ab254402** at 1/5000 dilution (0.01ug) (Both panels). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Dot Blot - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade

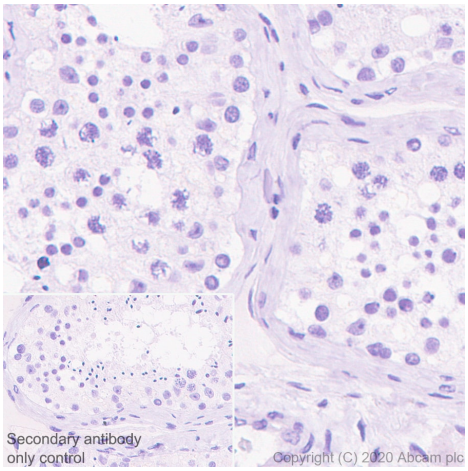
Dot blot analysis of Histone H3.3 (mutated G34 R) labeled with **ab254402** at 1/1000 dilution.

Lane 1: Histone H3.3 H3G34R peptide (aa28-40).
Lane 2: Histone H3.3 H3G34R peptide (aa26-36).
Lane 3: Histone H3.3 WT peptide (aa26-40).

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

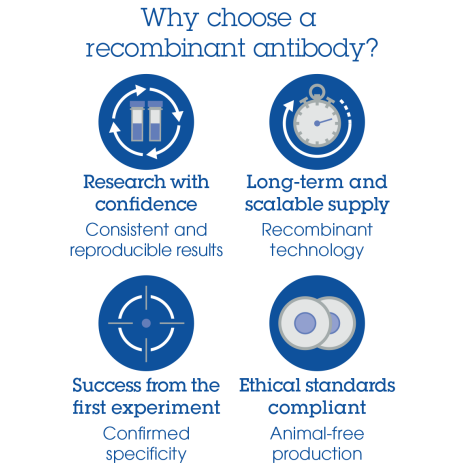


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling Histone H3.3 (mutated G34 R) with **ab254402** at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). **Negative control:** no staining on human testis. The section was incubated with **ab254402** for 30 mins at room temperature. 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 solution2) for 20 mins.



Human Histone H3 (Mutated K27M, K36M) + Histone H3.3 (Mutated G34W, G34V, G34R) Antibody Panel (ab274411)

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