

## Product Datasheet

# Histone H3 HIST1H3A/B/C/D/E/F/G/H/I/J Rabbit Polyclonal Antibody (orb614120)

<b>Catalog Number</b>	orb614120
<b>Category</b>	Antibodies
<b>Description</b>	Anti-Histone H3 HIST1H3A/B/C/D/E/F/G/H/I/J Antibody. Tested in ELISA, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
<b>Target</b>	Histone H3.1
<b>Clonality</b>	Polyclonal
<b>Species/Host</b>	Rabbit
<b>Isotype</b>	Rabbit IgG
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Human, Mouse, Rat
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	500 µg/ml
<b>Buffer/Preservatives</b>	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> .
<b>Reconstitution</b>	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.
<b>Purification</b>	Immunogen affinity purified.
<b>Immunogen</b>	E.coli-derived human Histone H3 recombinant protein (Position: Q56—R117).
<b>UniProt ID</b>	<b>P68431</b>

**Biorbyt Ltd.**

7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

Phone: [+44 \(0\)1223 859353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

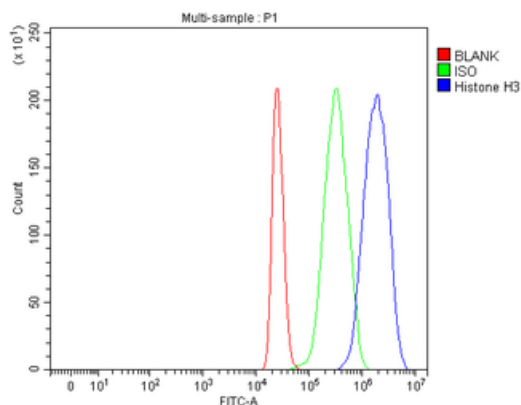
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United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

Phone: [+1 \(415\) 906-5211](tel:+1(415)906-5211) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

<b>MW</b>	17 kDa
<b>Tested applications</b>	ELISA, FC, ICC, IF, IHC, WB
<b>Dilution range</b>	Western blot, 0.1-0.25µg/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 5µg/ml, Human Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells, Mouse, Rat ELISA, 0.1-0.5µg/ml, -
<b>Specificity</b>	No cross reactivity with other proteins.
<b>Cross Reactivity</b>	No cross-reactivity with other proteins.
<b>Antibody Type</b>	Primary Antibody
<b>Storage</b>	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
<b>Note</b>	For research use only
<b>Expiration Date</b>	12 months from date of receipt.



Flow Cytometry analysis of C6 cells using anti-Histone H3 antibody. Overlay histogram showing C6 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Histone H3 Antibody (1 µg/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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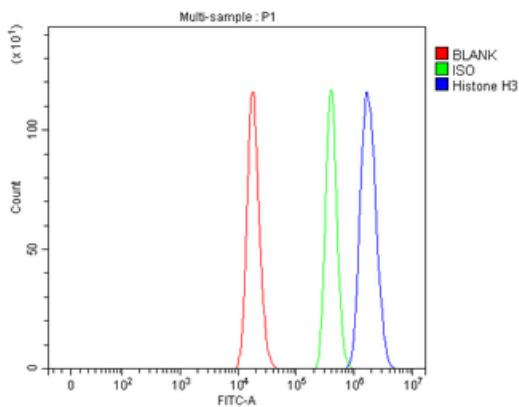
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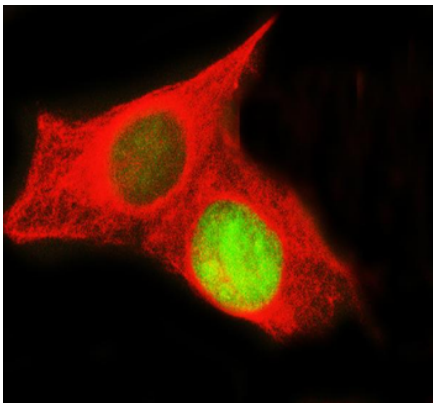
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Durham  
NC 27713  
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Flow Cytometry analysis of RAW264.7 cells using anti-Histone H3 antibody. Overlay histogram showing RAW264.7 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Histone H3 Antibody (1  $\mu\text{g}/1 \times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10  $\mu\text{g}/1 \times 10^6$  cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1  $\mu\text{g}/1 \times 10^6$ ) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



IF analysis of Histone H3 using anti-Histone H3 antibody and anti-Beta Tubulin antibody. Histone H3 was detected in immunocytochemical section of U2OS cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5  $\mu\text{g}/\text{mL}$  rabbit anti-Histone H3 Antibody and mouse anti-Beta Tubulin antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG and Cy3 Conjugated Goat Anti-Mouse IgG were used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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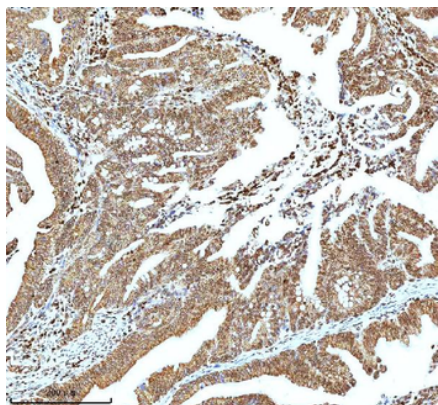
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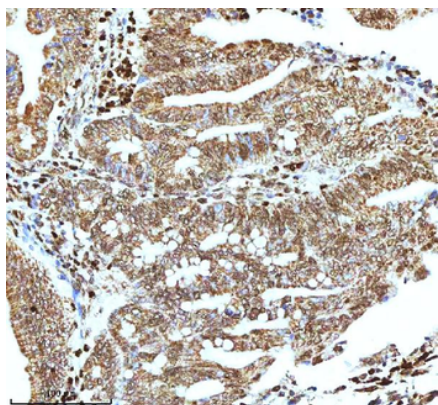
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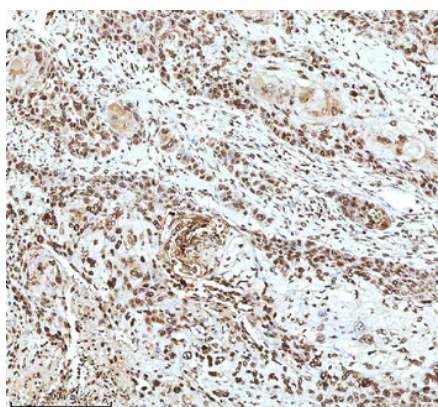
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IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of human colon adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



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IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of human esophageal squamous carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.

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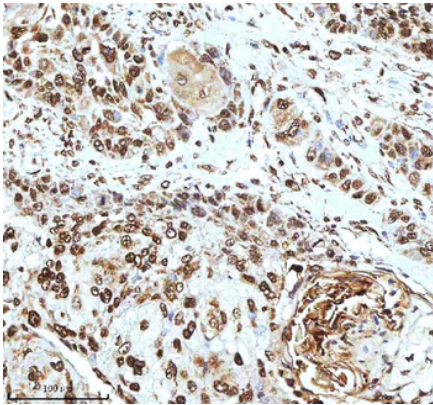
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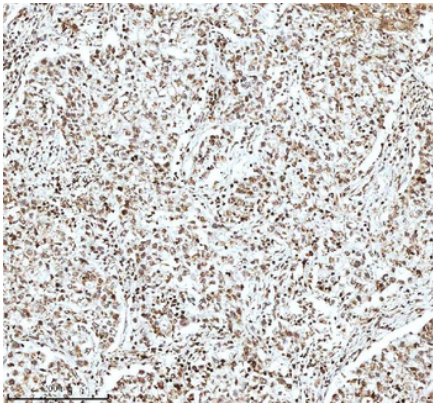
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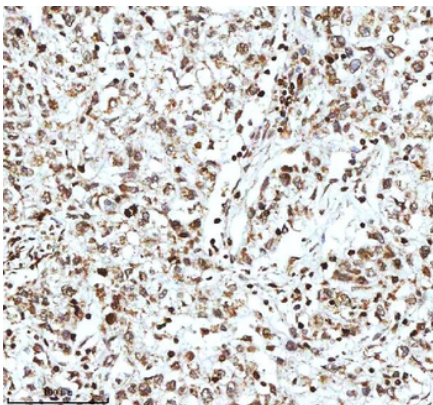
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IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of human esophageal squamous carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu$ g/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu$ g/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2  $\mu$ g/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.

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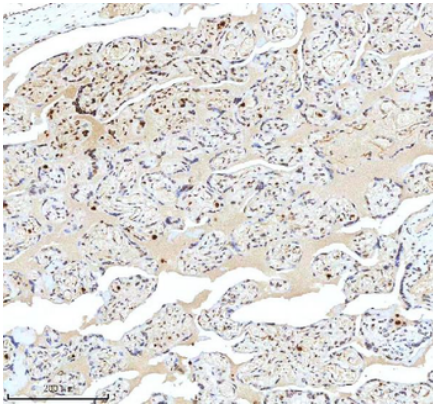
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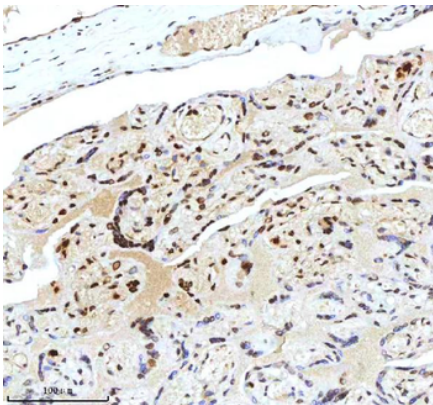
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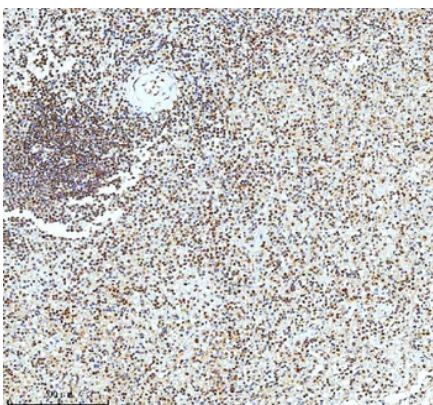
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IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μg/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



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IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of human spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μg/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.

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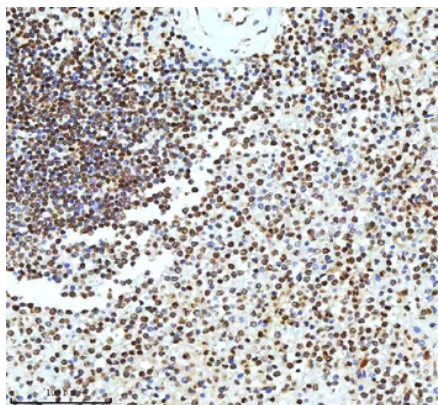
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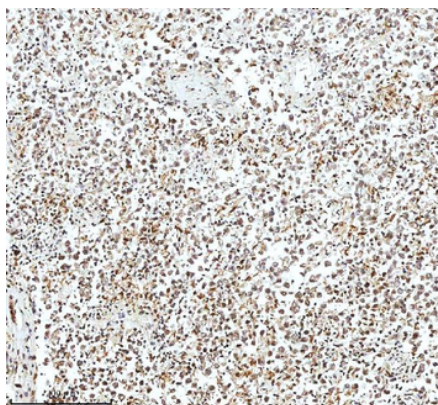
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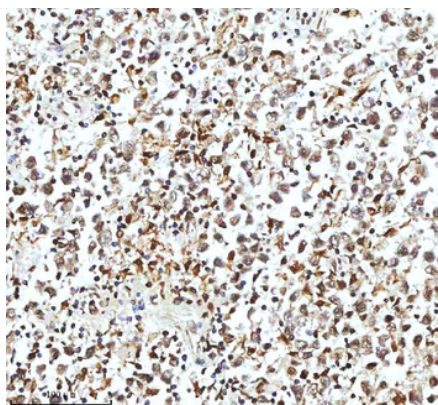
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IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of human spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of human testicular seminoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



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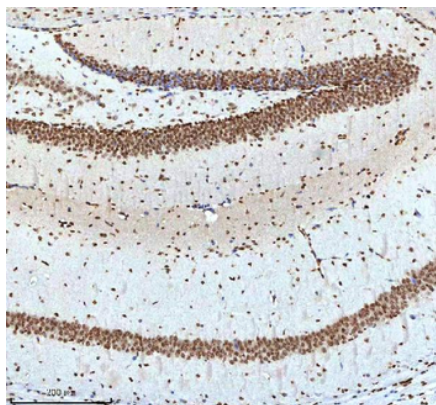
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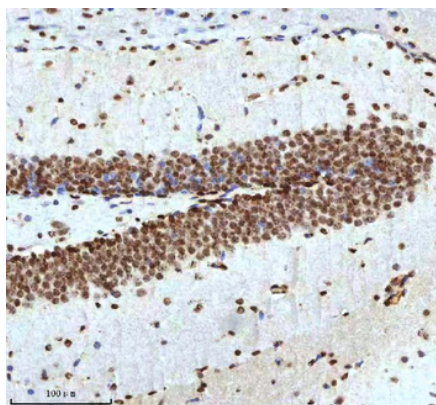
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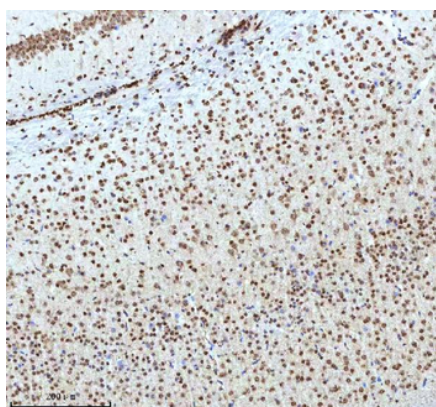
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IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μg/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



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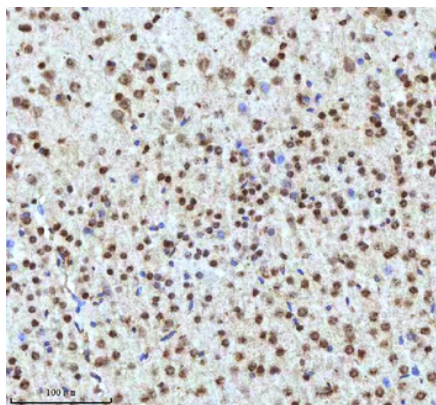
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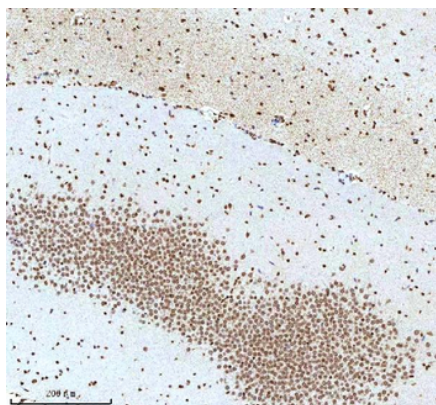
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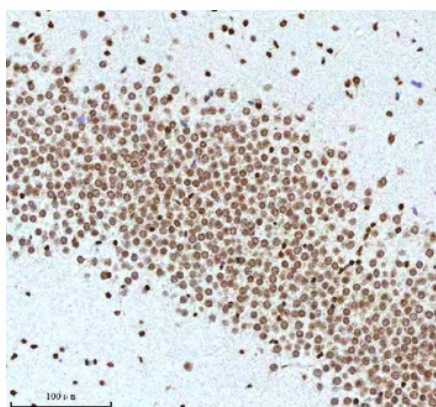
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IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μg/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



IHC analysis of Histone H3 using anti-Histone H3 antibody. Histone H3 was detected in a paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μg/ml rabbit anti-Histone H3 Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



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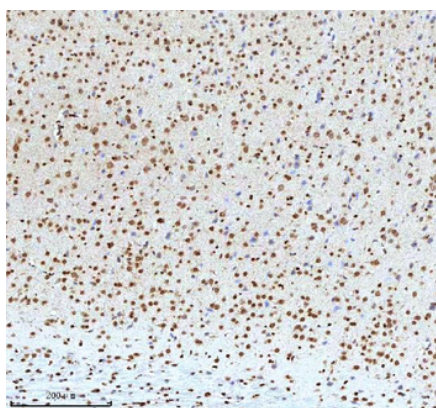
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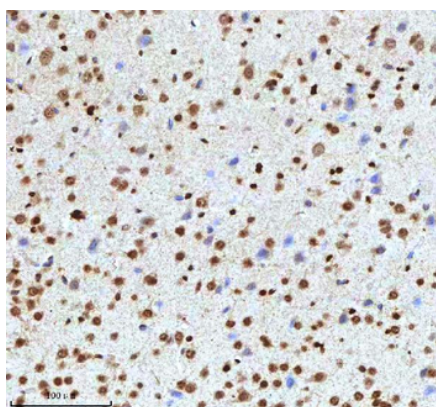
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**Biorbyt Ltd.**

7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

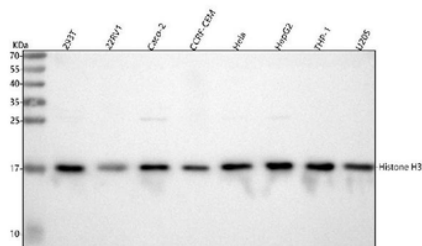
Phone: +44 (0)1223 859353 | Fax: +1 (415) 651-8558

**Biorbyt LLC**

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558



Western blot analysis of Histone H3 using anti-Histone H3 antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human 293T whole cell lysates, Lane 2: human 22RV1 whole cell lysates, Lane 3: human CACO-2 whole cell lysates, Lane 4: human CCRF-CEM whole cell lysates, Lane 5: human Hela whole cell lysates, Lane 6: human HepG2 whole cell lysates, Lane 7: human THP-1 whole cell lysates, Lane 8: human U2OS whole cell lysates. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-Histone H3 antigen affinity purified polyclonal antibody at 0.25  $\mu$ g/mL overnight at 4°C, then washed with TBS-0.1% Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for Histone H3 at approximately 17 kDa. The expected band size for Histone H3 is at 15 kDa.

**Biorbyt Ltd.**

7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

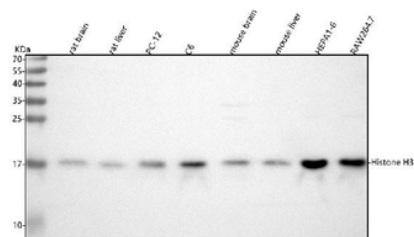
Phone: [+44 \(0\)1223 859353](tel:+44(0)1223859353) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)

**Biorbyt LLC**

68 TW Alexander Drive  
Research Triangle Park  
Durham  
NC 27713  
United States

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)

Phone: [+1 \(415\) 906-5211](tel:+1(415)906-5211) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)



Western blot analysis of Histone H3 using anti-Histone H3 antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: rat brain tissue lysates, Lane 2: rat liver tissue lysates, Lane 3: rat PC-12 whole cell lysates, Lane 4: rat C6 whole cell lysates, Lane 5: mouse brain tissue lysates, Lane 6: mouse liver tissue lysates, Lane 7: mouse HEPA1-6 whole cell lysates, Lane 8: mouse RAW264.7 whole cell lysates. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-Histone H3 antigen affinity purified polyclonal antibody at 0.25  $\mu\text{g}/\text{mL}$  overnight at 4°C, then washed with TBS-0.1% Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for Histone H3 at approximately 17 kDa. The expected band size for Histone H3 is at 15 kDa.

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7 Signet Court, Swann Road  
Cambridge  
CB5 8LA  
United Kingdom

Email: [info@biorbyt.com](mailto:info@biorbyt.com), [support@biorbyt.com](mailto:support@biorbyt.com)  
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Durham  
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Phone: [+1 \(415\) 906-5211](tel:+1(415)906-5211) | Fax: [+1 \(415\) 651-8558](tel:+1(415)651-8558)