NEUROMICS

Brg1 Data Sheet

Catalog Number:	GT15244	**Host:**	Goat
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Human
Immunogen Sequence:	Purified, *E. coli*-derived, recombinant human Brg1 Gln673-ASN774. Accession # P51532	**Format:**	Liquid 1mg/ml Solution in phosphate-buffered saline (PBS) with 5% Trehlose
Applications:	Immunohistochemistry - 5-15µg/mL Western Blot – 2ug/mL		
	Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Antibody can be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. *Avoid repeated freeze-thaw cycles.*		

Application Notes

Immunocytochemistry

Note: This protocol is optimized for cells grown on coverslips in a 6- or 24-well plate but can be adapted accordingly.

1. Wash the coverslips containing the fixed cells two times in 400 µL of wash buffer.
2. Block non-specific staining by adding 400 µL of blocking buffer and incubate for 45 minutes at room temperature.
3. Remove blocking buffer. No rinsing is necessary.
4. Dilute the antibody in dilution buffer to 5-10 µg/mL. For fluorescent ICC staining of cells, it is recommended to incubate at room temperature for 1 hour. Alternatively, incubate overnight at 2-8 °C.
   Note: Appropriate controls are critical for the accurate interpretation of IHC/ICC results. All IHC/ICC experiments should include a negative control using the incubation buffer with no primary antibody to identify non-specific staining of the secondary reagents. Additional controls can be employed to support the specificity of staining generated by the primary antibody.
5. Wash two times in 400 µL of wash buffer.
6. Dilute the secondary antibody in dilution buffer according to the manufacturer's instructions. Add 400 µL to the wells, and incubate at room temperature for 1 hour in the dark. From this step forward samples should be protected from light. Rinse two times in 400 µL of wash buffer.
7. Add 300 µL of the diluted DAPI solution to each well, and incubate 2-5 minutes at room temperature. DAPI binds to DNA and is a convenient nuclear counterstain. It has an absorption maximum at 358 nm and fluoresces blue at an emission maximum of 461 nm.
   Note: DAPI counterstain can obscure visualization of targets localized in cell nuclei.
8. Rinse once with PBS and once with water.
9. Carefully remove the coverslips from the wells and blot to remove any excess water. Dispense 1 drop of anti-fade mounting medium onto the microscope slide per coverslip. Mount the coverslip with the cells facing towards the microscope slide.
10. Visualize using a fluorescence microscope and filter sets appropriate for the label used. Slides can also be stored in a slide box at ≤ -20 °C for later examination.

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Western blot
Prepare total cell lysates by solubilizing cells in an appropriate sample buffer, such as 2X SDS sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue), at approximately 2x106-1x10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with 3-4 bursts of 5-10 seconds each.

1. Prepare the following solutions:

Blocking Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4, 0.5 M NaCl, 0.05% Tween 20	3% BSA in Blotting Buffer, Adjust pH to 7.4	0.5% BSA in Blotting Buffer, Adjust pH to 7.4

2. Transfer the electrophoresed proteins to a PVDF membrane and incubate for 1 hour at room temperature in Blocking Solution.
3. Incubate the membrane overnight at 4°C in Antibody Solution containing primary antibody.
4. Wash the membrane at room temperature for 30-60 minutes with 5 or more changes of Blotting Buffer.
5. Incubate the membrane for 1 hour at room temperature in Antibody Solution containing appropriate dilution of HRP-conjugated secondary antibody.
6. Wash the membrane for 30-60 minutes with 5 or more changes of Blotting Buffer.
7. Detect with Chemiluminescent Detection Substrate.
8. Expose to film and develop image.

Image: Brg1 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Goat Anti-Human Brg1 Antigen Affinity-purified Polyclonal Antibody at 10 µg/mL for 3 hours at room temperature. Cells were stained with 557-conjugated anti-Goat IgG Secondary Antibody (red, upper panel) and counterstained with DAPI (blue, lower panel). Specific staining was localized to nuclei.

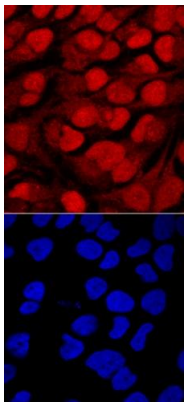
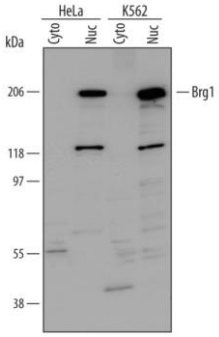


Image (right): Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and K562 human chronic myelogenous leukemia cell line, cytoplasmic and nuclear extracts. PVDF membrane was probed with 2 µg/mL Brg1 followed by HRP-conjugated Anti-Goat IgG Secondary Antibody. A specific band for Brg1 was detected at approximately 205 kDa (as indicated).



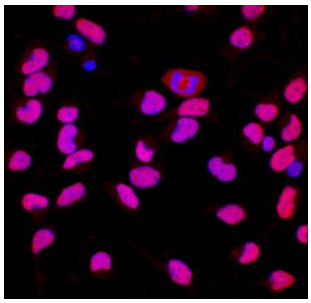


Image (left): Brg1 was detected in immersion fixed undifferentiated rat cortical stem cells using Goat Anti-Human Brg1 Antigen Affinity-purified Polyclonal Antibody at 10 µg/mL for 3 hours at room temperature. Cells were stained with 557-conjugated anti-Goat IgG Secondary Antibody (red) and counterstained with DAPI (blue). Specific staining was localized to nuclei.

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