

## Product Datasheet

### Anti-Prohibitin/PHB Antibody (orb745921)

<b>Description</b>	Anti-Prohibitin/PHB Antibody. Tested in ELISA, Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
<b>Species/Host</b>	Rabbit
<b>Reactivity</b>	Human, Mouse, Rat
<b>Conjugation</b>	Unconjugated
<b>Tested Applications</b>	ELISA, FC, ICC, IF, IHC, WB
<b>Immunogen</b>	E.coli-derived human Prohibitin/PHB recombinant protein (Position: M1-I257).
<b>Form/Appearance</b>	Lyophilized
<b>Concentration</b>	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
<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>Application notes</b>	Western blot, 0.25-0.5µg/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human ELISA, 0.1-0.5µg/ml, -. Add 0.2ml of distilled water will yield a concentration of 500ug/ml
<b>Isotype</b>	Rabbit IgG
<b>Clonality</b>	Polyclonal
<b>Antibody Type</b>	Primary Antibody
<b>MW</b>	30 kDa
<b>Uniprot ID</b>	<b>P35232</b>

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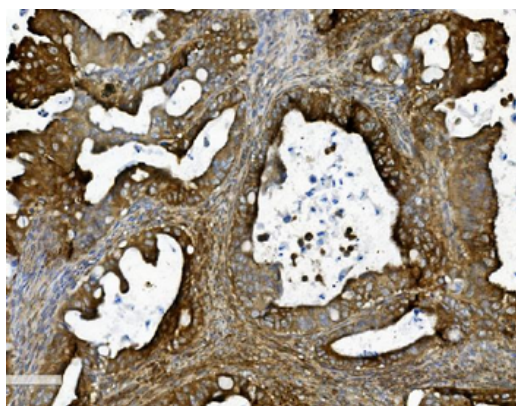
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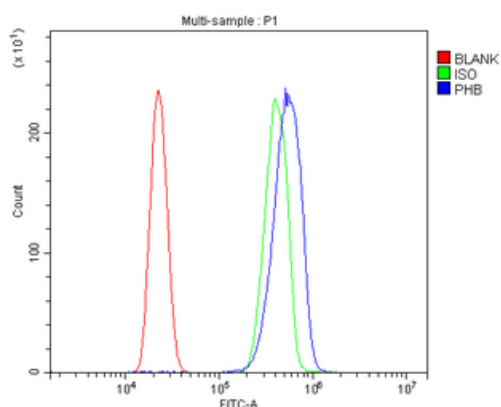
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**Expiration Date**

12 months from date of receipt.



IHC analysis of Prohibitin/PHB using anti-Prohibitin/PHB antibody. Prohibitin/PHB was detected in paraffin-embedded section of human ovarian 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 µg/ml rabbit anti-Prohibitin/PHB Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



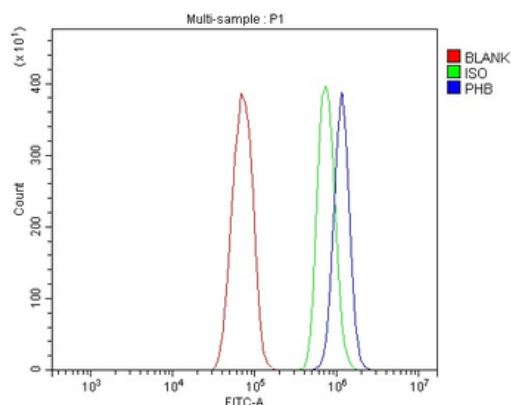
Flow Cytometry analysis of HL-60 cells using anti-Prohibitin/PHB antibody. Overlay histogram showing HL-60 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Prohibitin/PHB 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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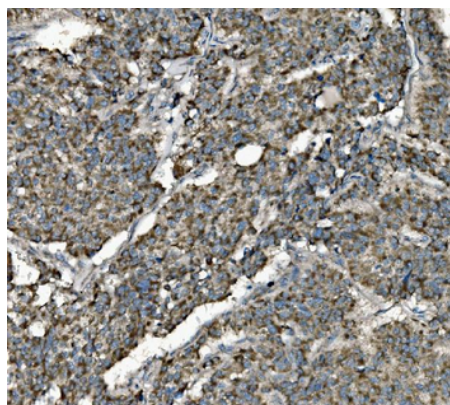
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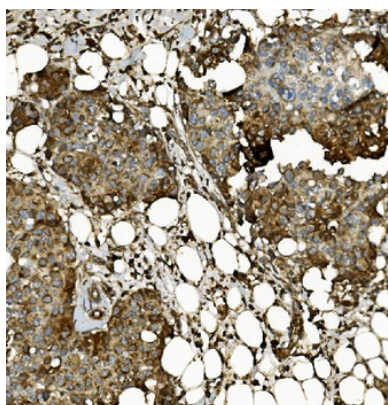
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Flow Cytometry analysis of RH35 cells using anti-Prohibitin/PHB antibody. Overlay histogram showing RH35 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Prohibitin/PHB 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.



IHC analysis of Prohibitin/PHB using anti-Prohibitin/PHB antibody. Prohibitin/PHB was detected in paraffin-embedded section of human adrenocortical adenoma 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  $\mu\text{g}/\text{ml}$  rabbit anti-Prohibitin/PHB Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



IHC analysis of Prohibitin/PHB using anti-Prohibitin/PHB antibody. Prohibitin/PHB was detected in paraffin-embedded section of human breast 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  $\mu\text{g}/\text{ml}$  rabbit anti-Prohibitin/PHB Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

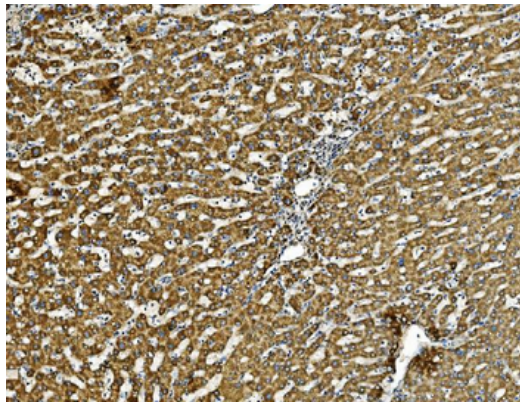
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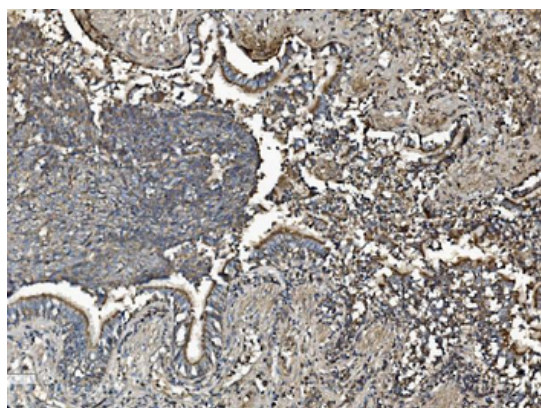
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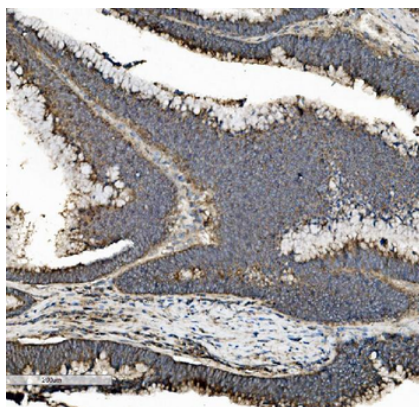




IHC analysis of Prohibitin/PHB using anti-Prohibitin/PHB antibody. Prohibitin/PHB was detected in paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Prohibitin/PHB Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



IHC analysis of Prohibitin/PHB using anti-Prohibitin/PHB antibody. Prohibitin/PHB was detected in 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 µg/ml rabbit anti-Prohibitin/PHB Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



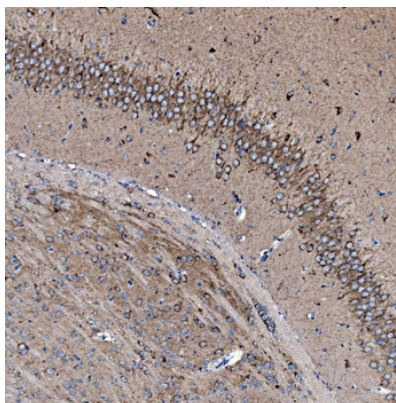
IHC analysis of Prohibitin/PHB using anti-Prohibitin/PHB antibody. Prohibitin/PHB was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Prohibitin/PHB Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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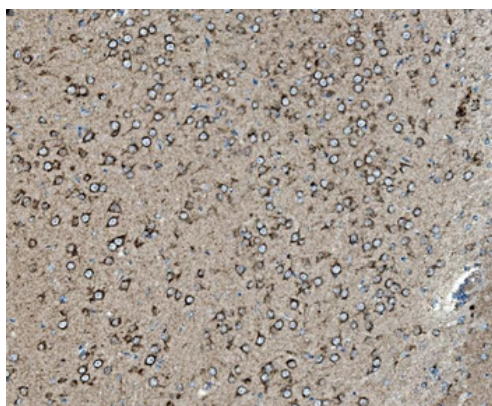
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IHC analysis of Prohibitin/PHB using anti-Prohibitin/PHB antibody. Prohibitin/PHB was detected in paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Prohibitin/PHB Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



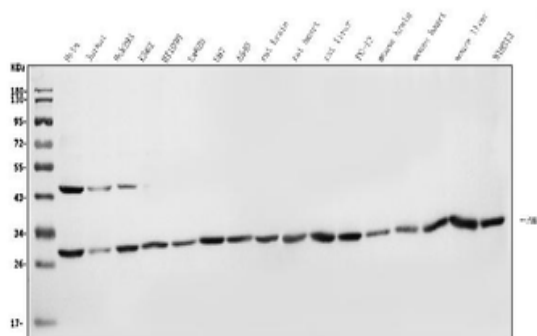
IHC analysis of Prohibitin/PHB using anti-Prohibitin/PHB antibody. Prohibitin/PHB was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Prohibitin/PHB Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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Western blot analysis of Prohibitin/PHB using anti-Prohibitin/PHB 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 Hela whole cell lysates, Lane 2: human Jurkat whole cell lysates, Lane 3: human Hek293 whole cell lysates, Lane 4: human K562 whole cell lysates, Lane 5: human HT1080 whole cell lysates, Lane 6: human Sw620 whole cell lysates, Lane 7: human U87 whole cell lysates, Lane 8: human A549 whole cell lysates, Lane 9: rat brain tissue lysates, Lane 10: rat heart tissue lysates, Lane 11: rat liver tissue lysates, Lane 12: rat PC-12 whole cell lysates, Lane 13: mouse brain tissue lysates, Lane 14: mouse heart tissue lysates, Lane 15: mouse liver tissue lysates, Lane 16: mouse NIH/3T3 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-Prohibitin/PHB antigen affinity purified polyclonal antibody at 0.5 µg/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 Prohibitin/PHB at approximately 28-30 KD. The expected band size for Prohibitin/PHB is at 28-30 KD.

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