

## Product Datasheet

# Nicotinic Acetylcholine Receptor alpha 5/CHRNA5 Rabbit Polyclonal Antibody (orb389469)

<b>Catalog Number</b>	orb389469
<b>Category</b>	Antibodies
<b>Description</b>	Nicotinic Acetylcholine Receptor alpha 5/CHRNA5 Rabbit Polyclonal Antibody
<b>Target</b>	Neuronal acetylcholine receptor subunit alpha-5
<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>	Adding 0.2 ml of distilled water will yield a concentration of 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> , 0.05mg Na <sub>3</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>	A synthetic peptide corresponding to a sequence at the N-terminus of human CHRNA5, different from the related mouse sequence by five amino acids, and from the related rat sequence by four amino acids.
<b>UniProt ID</b>	<b>P30532</b>

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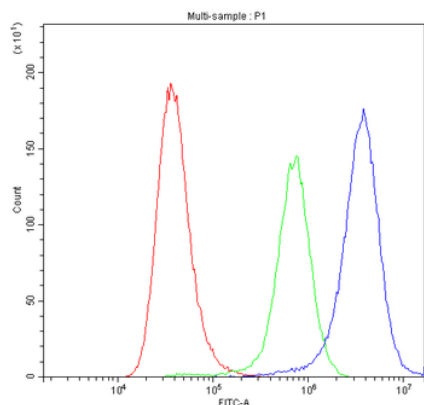
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<b>MW</b>	53 kDa
<b>Tested applications</b>	FC, ICC, IHC, WB
<b>Dilution range</b>	Immunohistochemistry (Paraffin-embedded Section), 0.5-1µg/ml, Human, Mouse, Rat Western blot, 0.1-0.5µg/ml, Human, Rat Immunohistochemistry (Frozen Section), 0.5-1µg/ml, Human Immunocytochemistry, 0.5-1µg/ml, Human Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells, Human
<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 A549 cells using anti-CHRNA5 antibody. Overlay histogram showing A549 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-CHRNA5 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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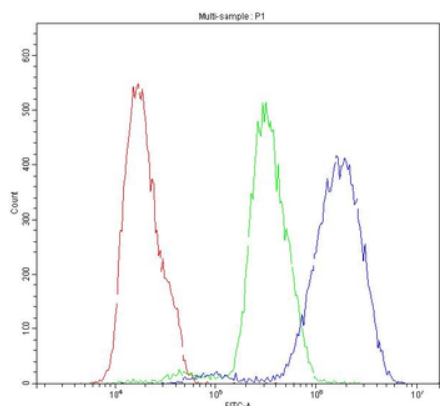
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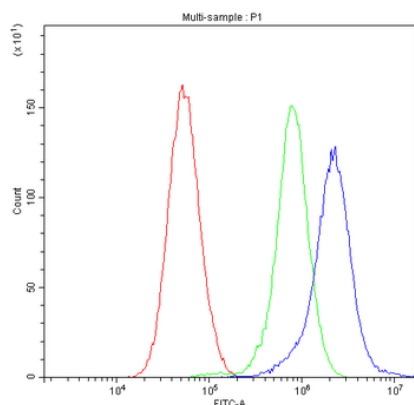
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Flow Cytometry analysis of MCF-7 cells using anti-CHRNA5 antibody. Overlay histogram showing MCF-7 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-CHRNA5 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.



Flow Cytometry analysis of U251 cells using anti-CHRNA5 antibody. Overlay histogram showing U251 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-CHRNA5 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.

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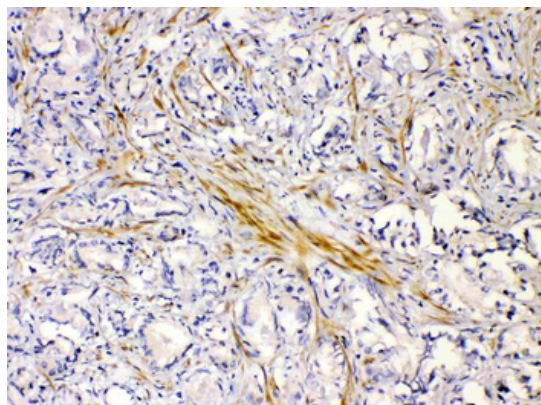
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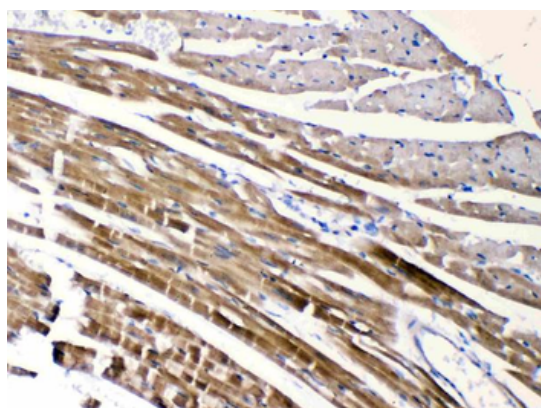
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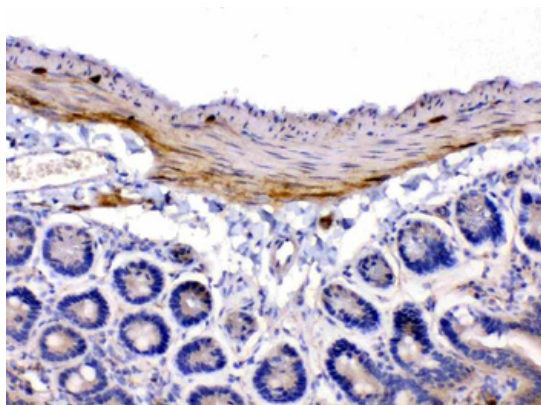
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IHC analysis of CHRNA5 using anti-CHRNA5 antibody. CHRNA5 was detected in paraffin-embedded section of human prostatic cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu$ g/ml rabbit anti-CHRNA5 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 CHRNA5 using anti-CHRNA5 antibody. CHRNA5 was detected in paraffin-embedded section of mouse cardiac muscle tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu$ g/ml rabbit anti-CHRNA5 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 CHRNA5 using anti-CHRNA5 antibody. CHRNA5 was detected in paraffin-embedded section of mouse intestine tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu$ g/ml rabbit anti-FBXL4 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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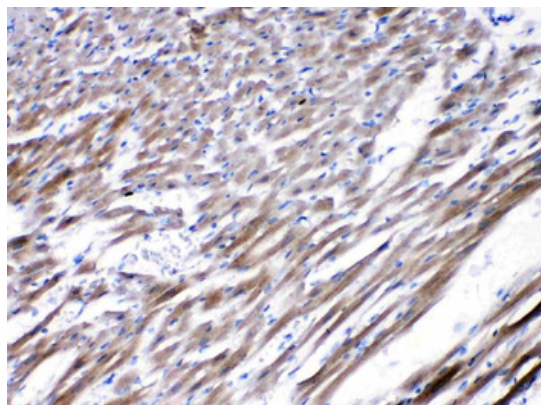
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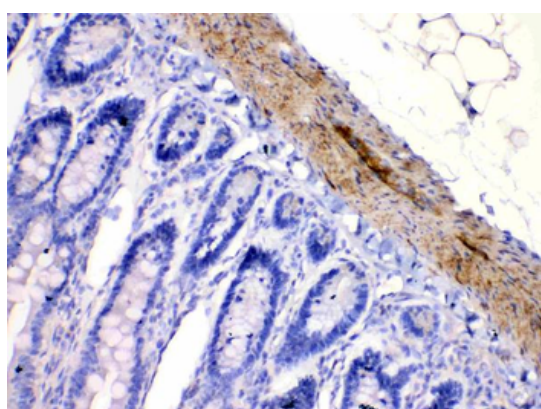
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IHC analysis of CHRNA5 using anti-CHRNA5 antibody. CHRNA5 was detected in paraffin-embedded section of rat cardiac muscle tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu$ g/ml rabbit anti-FBXL4 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 CHRNA5 using anti-CHRNA5 antibody. CHRNA5 was detected in paraffin-embedded section of rat intestine tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu$ g/ml rabbit anti-CHRNA5 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 CHRNA5 using anti-CHRNA5 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 50 ug of sample under reducing conditions. lane 1: rat skeletal muscle tissue lysates, lane 2: HEPG2 whole cell lysates. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA 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-CHRNA5 antigen affinity purified polyclonal antibody at 0.5  $\mu\text{g}/\text{mL}$  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 1:10000 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 CHRNA5 at approximately 53KD. The expected band size for CHRNA5 is at 53KD.

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