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antibody and ELISA experts

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Anti-REA/PHB2 Antibody Picoband™ (monoclonal, 2B5)

Catalog Number: M03315

About PHB2

PHB2 (Prohibitin 2), also called Repressor of Estrogen Receptor Activity (REA), is a protein that in humans is encoded by the PHB2 gene. The International Radiation Hybrid Mapping Consortium mapped the PHB2 gene to chromosome 12. Montano et al. (1999) showed that REA enhanced the potency of a dominant-negative ER-alpha mutant and antiestrogens as suppressors of ER-alpha activity in Chinese hamster ovary cells. When coexpressed with wildtype ER-alpha or ER-beta (ESR2), REA suppressed activation of a reporter gene in a dose-dependent manner. REA had no effect on reporter activity in the absence of liganded ER, and it had no effect on the transcriptional activities of other hormone receptors. Mutation analysis showed that an N-terminal domain and a central domain of REA were required for its repressor activity.

Overview

Product Name	Anti-REA/PHB2 Antibody Picoband™ (monoclonal, 2B5)
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-REA/PHB2 Antibody Picoband™ (monoclonal, 2B5) catalog # M03315. Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IHC, WB
Clonality	Monoclonal 2B5
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Storage Instructions	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.
Host	Mouse
Uniprot ID	Q99623

Technical Details

Immunogen	E.coli-derived human REA/PHB2 recombinant protein (Position: M1-K299).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG2b
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.

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Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.1-0.25ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human, Mouse, Rat Flow Cytometry, 1-3ug/1x10^6 cells, Human

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Anti-REA/PHB2 Antibody Picoband™ (monoclonal, 2B5) (M03315) Images

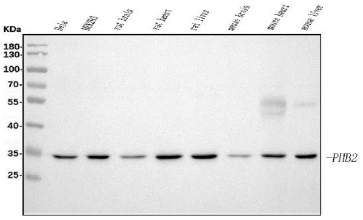

Figure 1. Western blot analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human HEK293 whole cell lysates,
Lane 3: rat brain tissue lysates,
Lane 4: rat heart tissue lysates,
Lane 5: rat liver tissue lysates,
Lane 6: mouse brain tissue lysates,
Lane 7: mouse heart tissue lysates,
Lane 8: mouse liver tissue 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 mouse anti-REA/PHB2 antigen affinity purified monoclonal antibody (Catalog # M03315) at 0.25 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse 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 (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for REA/PHB2 at approximately 32KD. The expected band size for REA/PHB2 is at 32KD.

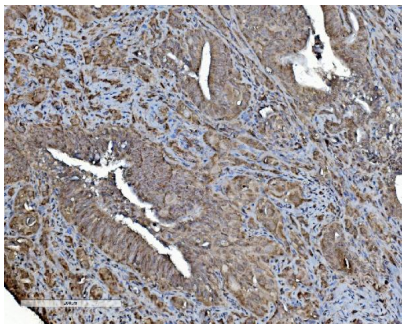

Figure 2. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of human gallbladder adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-REA/PHB2 Antibody (M03315) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

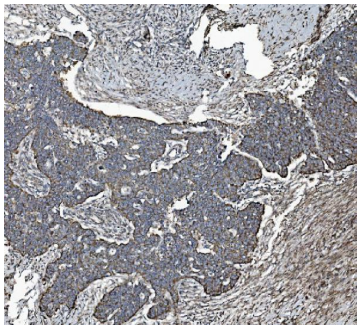

Figure 3. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of human ovarian serous adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-REA/PHB2 Antibody (M03315) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30

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minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

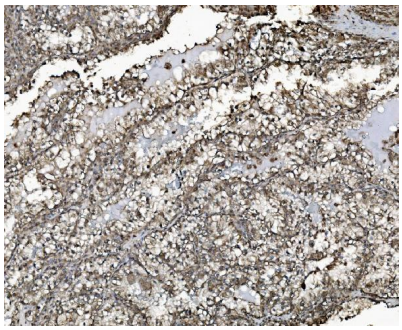

Figure 4. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of human renal clear cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-REA/PHB2 Antibody (M03315) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

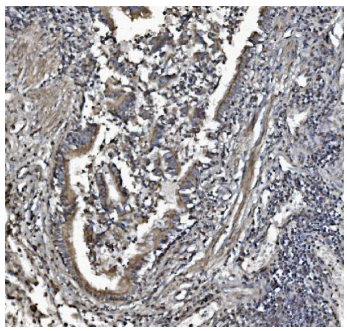

Figure 5. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-REA/PHB2 Antibody (M03315) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

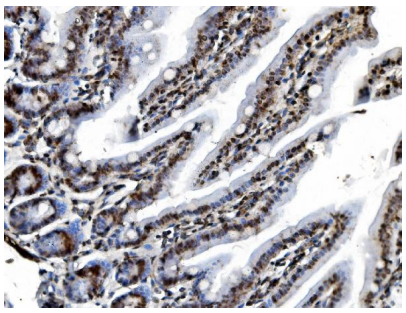

Figure 6. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of mouse colon tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-REA/PHB2 Antibody (M03315) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

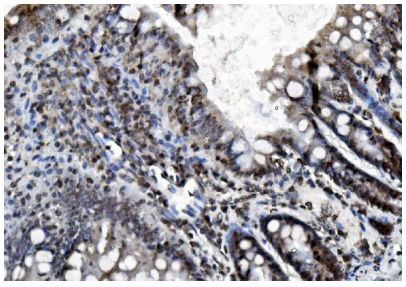

Figure 7. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of rat colon tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml mouse anti-REA/PHB2 Antibody (M03315) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

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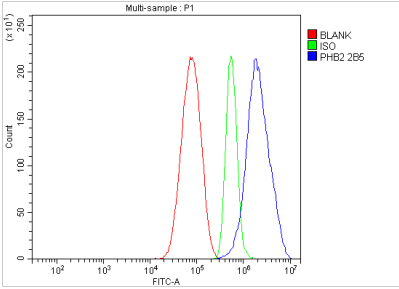

Figure 8. Flow Cytometry analysis of HepG2 cells using anti-REA/PHB2 antibody (M03315).
Overlay histogram showing HepG2 cells stained with M03315 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-REA/PHB2 Antibody (M03315, 1ug/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.