10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

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

Catalog # ABO15050

Specification

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

Application	**WB, IHC, FC**
Primary Accession	Q99623
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-REA/PHB2 Antibody Picoband™ (monoclonal, 2B5) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

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

Gene ID 11331

Other Names
Prohibitin-2, B-cell receptor-associated protein BAP37, D-prohibitin, Repressor of estrogen receptor activity, PHB2 {ECO:0000312|EMBL:AAH14766.1, ECO:0000312|HGNC:HGNC:30306}

Calculated MW
32 kDa KDa

Application Details
Western blot, 0.1-0.25 µg/ml, Human, Mouse, Rat
 Immunohistochemistry (Paraffin-embedded Section), 2-5 µg/ml, Human, Mouse, Rat
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.

Immunogen
E.coli-derived human REA/PHB2 recombinant protein (Position: M1-K299).

Purification
Immunogen affinity purified.

Storage	**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.**

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

Name PHB2 {ECO:0000312|EMBL:AAH14766.1, ECO:0000312|HGNC:HGNC:30306}

Function
Protein with pleiotropic attributes mediated in a cell- compartment- and tissue-specific manner, which include the plasma membrane-associated cell signaling functions, mitochondrial chaperone, and transcriptional co-regulator of transcription factors and sex steroid hormones in the nucleus.

Cellular Location
Mitochondrion inner membrane. Cytoplasm. Nucleus. Cell membrane Note=Localizes within both nucleus and cytoplasm in proliferative primary myoblasts and mostly within the nucleus of differentiated primary myoblasts. [Isoform 2]: Mitochondrion inner membrane

Tissue Location
Expressed in myoblasts.

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

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

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

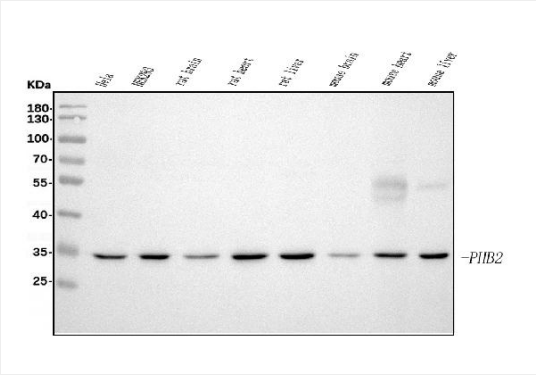

Figure 1. Western blot analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: human Hela 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 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-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.

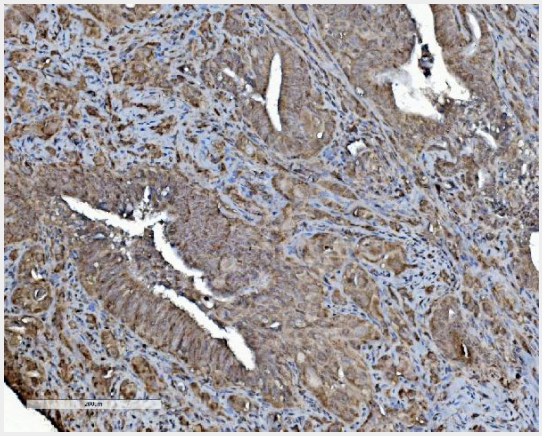

Figure 2. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of human gallbladder adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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.

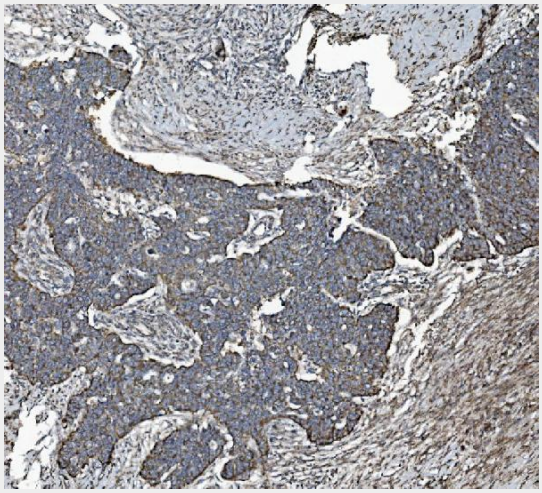

Figure 3. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of human ovarian serous adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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.

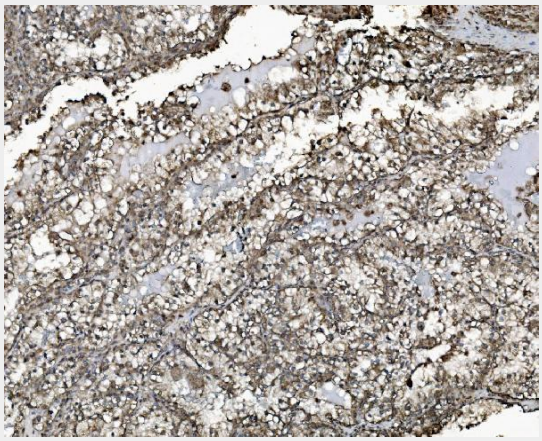

Figure 4. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of human renal clear cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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.

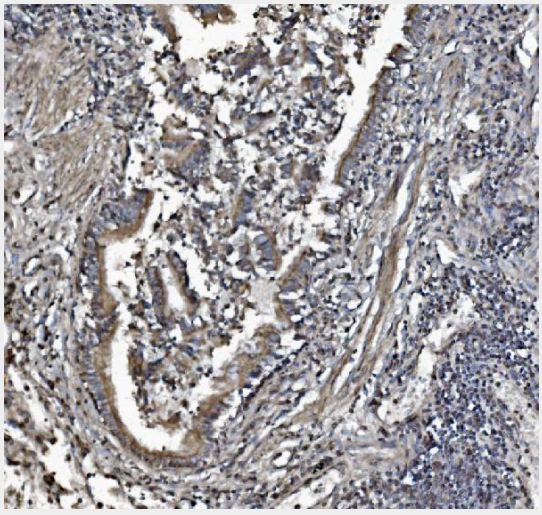

Figure 5. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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.

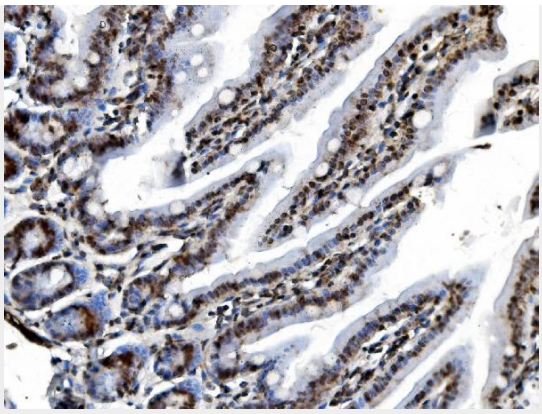

Figure 6. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of mouse colon tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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.

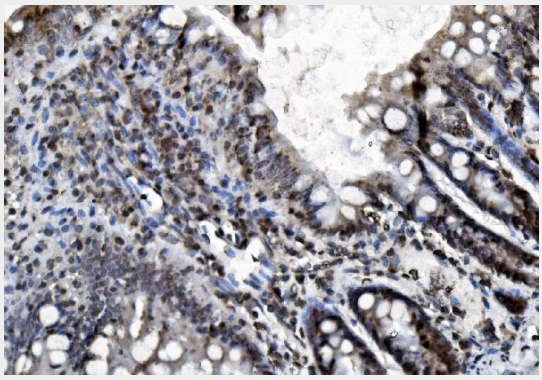

Figure 7. IHC analysis of REA/PHB2 using anti-REA/PHB2 antibody (M03315).
REA/PHB2 was detected in paraffin-embedded section of rat colon tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/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.

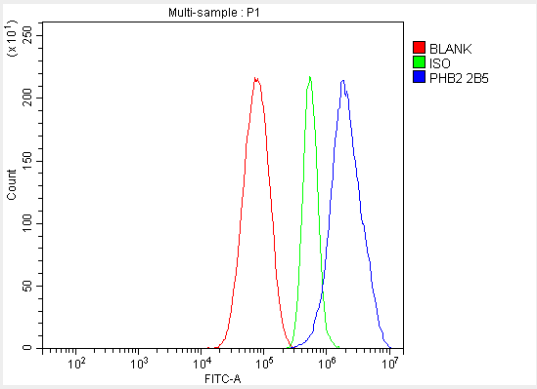

Figure 8. Flow Cytometry analysis of HepG2 cells using anti-REA/PHB2 antibody (M03315).
Overlay histogram showing HepG2 cells stained with M03315 (Blue line).The cells were blocked

with 10% normal goat serum. And then incubated with mouse anti-REA/PHB2 Antibody (M03315, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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

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.