10320 Camino Santa Fe, Suite G
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Anti-YWHAE Antibody Picoband™ (monoclonal, 3G11G2)

Catalog # ABO16243

Specification

Anti-YWHAE Antibody Picoband™ (monoclonal, 3G11G2) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	P62258
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-YWHAE Antibody Picoband™ (monoclonal, 3G11G2) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution
Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Anti-YWHAE Antibody Picoband™ (monoclonal, 3G11G2) - Additional Information

Gene ID 7531

Other Names
14-3-3 protein epsilon, 14-3-3E, YWHAE

Calculated MW
29 kDa KDa

Application Details
Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat
 Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human, Rat
 Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6 cells, Mouse, Rat

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

Immunogen
E.coli-derived human YWHAE recombinant protein (Position: M1-Q255).

Purification
Immunogen affinity purified.

Storage	**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 freezing and thawing.**

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Anti-YWHAE Antibody Picoband™ (monoclonal, 3G11G2) - Protein Information

Name YWHAE

Function
Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways (PubMed:21189250). Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif (PubMed:35343654). Binding generally results in the modulation of the activity of the binding partner (By similarity). Positively regulates phosphorylated protein HSF1 nuclear export to the cytoplasm (PubMed:12917326). Plays a positive role in the antiviral signaling pathway upstream of TBK1 via interaction with RIGI (PubMed:37555661). Mechanistically, directs RIGI redistribution from the cytosol to mitochondrial associated membranes where it mediates MAVS-dependent innate immune signaling during viral infection (PubMed:22607805). Plays a role in proliferation inhibition and cell cycle arrest by exporting HNRNPC from the nucleus to the cytoplasm to be degraded by ubiquitination (PubMed:37599448).

Cellular Location
Nucleus. Cytoplasm Melanosome Note=Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Anti-YWHAE Antibody Picoband™ (monoclonal, 3G11G2) - 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-YWHAE Antibody Picoband™ (monoclonal, 3G11G2) - Images

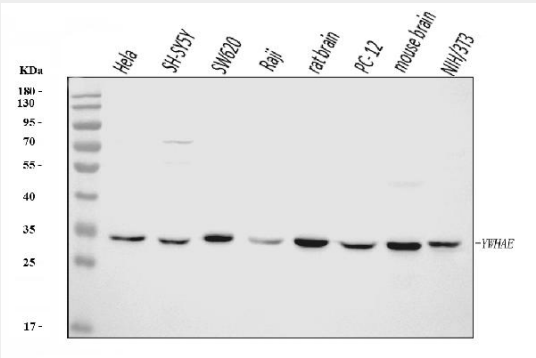

Figure 1. Western blot analysis of YWHAE using anti-YWHAE antibody (M01687-2).

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 SH-SY5Y whole cell lysates,
Lane 3: human SW620 whole cell lysates,
Lane 4: human Raji whole cell lysates,
Lane 5: rat brain tissue lysates,
Lane 6: rat PC-12 whole cell lysates,
Lane 7: mouse brain tissue lysates,
Lane 8: 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 mouse anti-YWHAE antigen affinity purified monoclonal antibody (Catalog # M01687-2) 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-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 YWHAE at approximately 29 kDa. The expected band size for YWHAE is at 29 kDa.

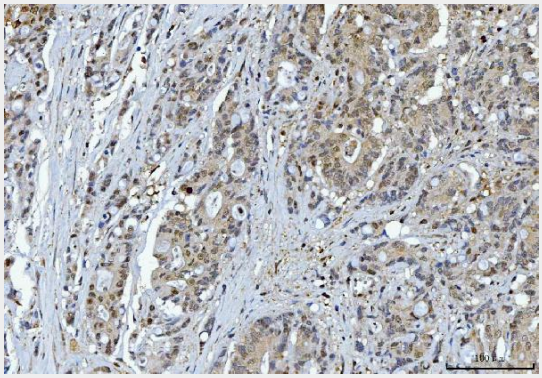

Figure 2. IHC analysis of YWHAE using anti-YWHAE antibody (M01687-2).
YWHAE was detected in a paraffin-embedded section of human colorectal adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-YWHAE Antibody (M01687-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

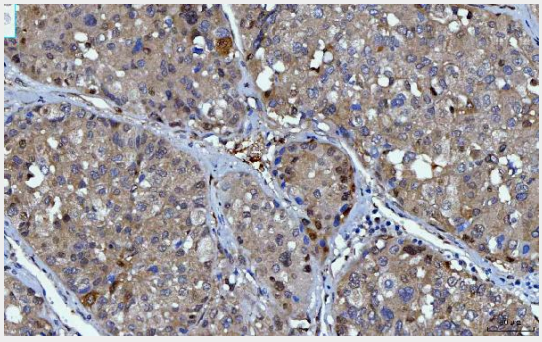

Figure 3. IHC analysis of YWHAE using anti-YWHAE antibody (M01687-2).
YWHAE was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml

mouse anti-YWHAE Antibody (M01687-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

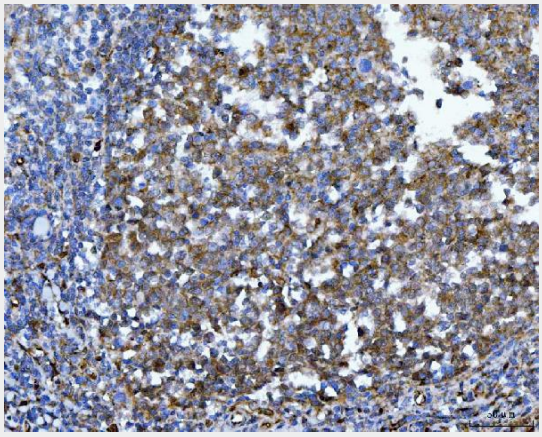

Figure 4. IHC analysis of YWHAE using anti-YWHAE antibody (M01687-2).
YWHAE was detected in a paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-YWHAE Antibody (M01687-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

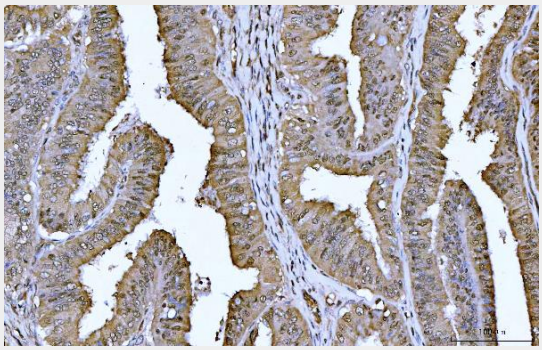

Figure 5. IHC analysis of YWHAE using anti-YWHAE antibody (M01687-2).
YWHAE was detected in a paraffin-embedded section of human endometrial cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-YWHAE Antibody (M01687-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

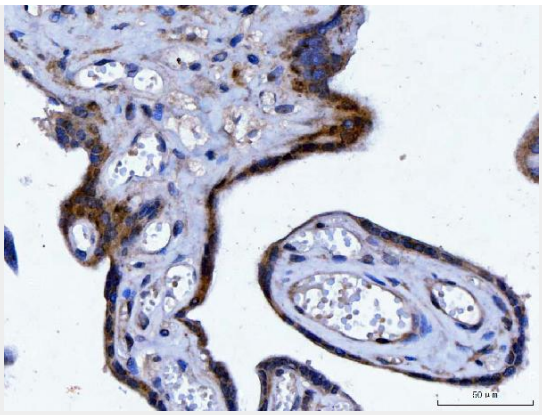

Figure 6. IHC analysis of YWHAE using anti-YWHAE antibody (M01687-2).
YWHAE was detected in a paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-YWHAE Antibody (M01687-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

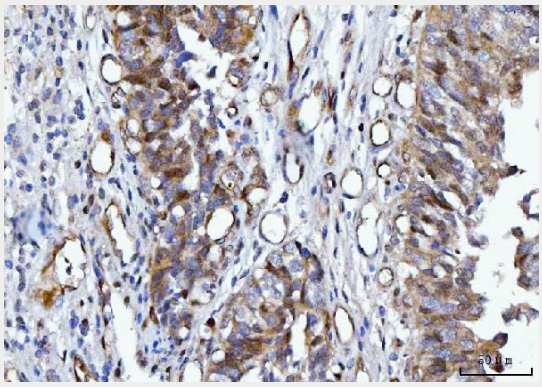

Figure 7. IHC analysis of YWHAE using anti-YWHAE antibody (M01687-2).
YWHAE was detected in a paraffin-embedded section of human bladder epithelial carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-YWHAE Antibody (M01687-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

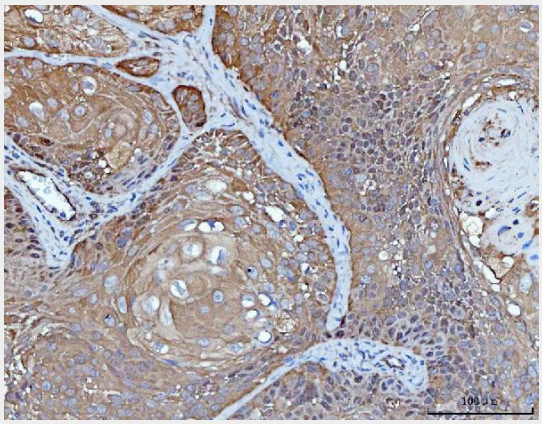

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Figure 8. IHC analysis of YWHAE using anti-YWHAE antibody (M01687-2).
YWHAE was detected in a paraffin-embedded section of human laryngeal squamous cell carcinomas tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-YWHAE Antibody (M01687-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

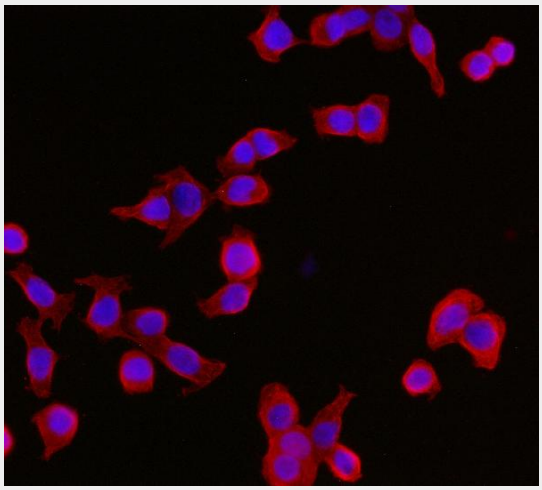

Figure 9. IF analysis of YWHAE using anti-YWHAE antibody (M01687-2).
YWHAE was detected in an immunocytochemical section of Caco-2 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-YWHAE Antibody (M01687-2) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Mouse IgG (BA1141) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

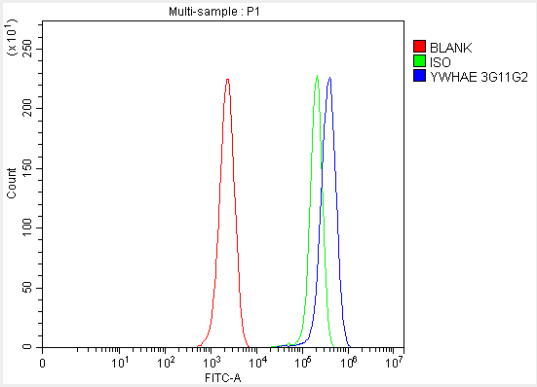

Figure 10. Flow Cytometry analysis of ANA-1 cells using anti-YWHAE antibody (M01687-2).
Overlay histogram showing ANA-1 cells stained with M01687-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-YWHAE Antibody (M01687-2, 1 µg/1x10^6 cells) for 30 min 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.

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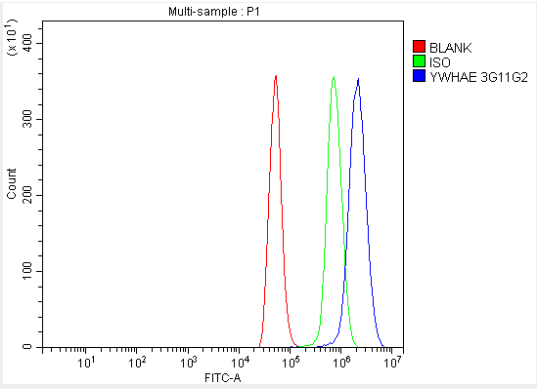

Figure 11. Flow Cytometry analysis of NRK cells using anti-YWHAE antibody (M01687-2).
Overlay histogram showing NRK cells stained with M01687-2 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-YWHAE Antibody (M01687-2, 1 µg/1x10^6 cells) for 30 min at 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.

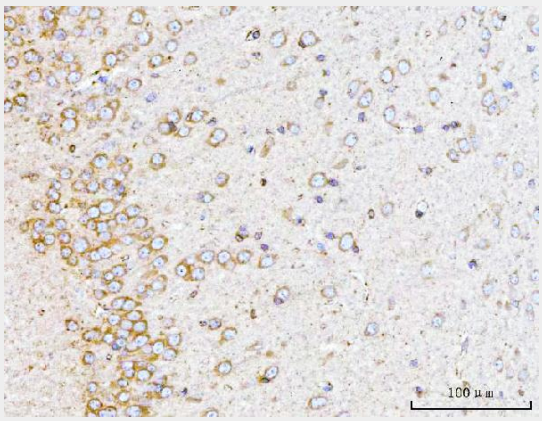

Figure 12. IHC analysis of YWHAE using anti-YWHAE antibody (M01687-2).
YWHAE was detected in a paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-YWHAE Antibody (M01687-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

Anti-YWHAE Antibody Picoband™ (monoclonal, 3G11G2) - Background

14-3-3 protein epsilon is a protein that in humans is encoded by the YWHAE gene. This gene product belongs to the 14-3-3 family of proteins which mediate signal transduction by binding to phosphoserine-containing proteins. This highly conserved protein family is found in both plants and mammals, and this protein is 100% identical to the mouse ortholog. It interacts with CDC25 phosphatases, RAF1 and IRS1 proteins, suggesting its role in diverse biochemical activities related to signal transduction, such as cell division and regulation of insulin sensitivity. It has also been implicated in the pathogenesis of small cell lung cancer. Two transcript variants, one protein-coding and the other non-protein-coding, have been found for this gene.