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Anti-COMT Antibody Picoband™ (monoclonal, 15C10)

Catalog # ABO14887

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P21964
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-COMT Antibody Picoband™ (monoclonal, 15C10) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	1312
Other Names	Catechol O-methyltransferase, 2.1.1.6, COMT (HGNC:2228)
Calculated MW	30037
Application Details	Western blot, 0.1-0.5 □g/ml, Human,Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml, Human, Mouse, Rat, By Heat Immunocytochemistry/Immunofluorescence, 2 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Subcellular Localization	Cytoplasm. Cell membrane. Single-pass type II membrane protein. Extracellular side.
Tissue Specificity	Brain, liver, placenta, lymphocytes and erythrocytes.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 15C10
Immunogen	E.coli-derived human COMT recombinant protein (Position: G52-P271). Human COMT shares 81.9% and 81% amino acid (aa) sequence identity with mouse and rat COMT, respectively.
Cross Reactivity	No cross-reactivity with other proteins.

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.

Protein Information

Name	COMT (HGNC:2228)
Function	Catalyzes the O-methylation, and thereby the inactivation, of catecholamine neurotransmitters and catechol hormones. Also shortens the biological half-lives of certain neuroactive drugs, like L-DOPA, alpha-methyl DOPA and isoproterenol.
Cellular Location	[Isoform Soluble]: Cytoplasm
Tissue Location	Brain, liver, placenta, lymphocytes and erythrocytes

Background

Catechol O-methyltransferase, also called COMT, is one of the major mammalian enzymes involved in the metabolic degradation of catecholamines. This gene is mapped to 22q11.21. Catechol-O-methyltransferase catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines, including the neurotransmitters dopamine, epinephrine, and norepinephrine. This O-methylation results in one of the major degradative pathways of the catecholamine transmitters. In addition to its role in the metabolism of endogenous substances, COMT is important in the metabolism of catechol drugs used in the treatment of hypertension, asthma, and Parkinson disease. COMT is found in two forms in tissues, a soluble form (S-COMT) and a membrane-bound form (MB-COMT). The differences between S-COMT and MB-COMT reside within the N-termini.

Images

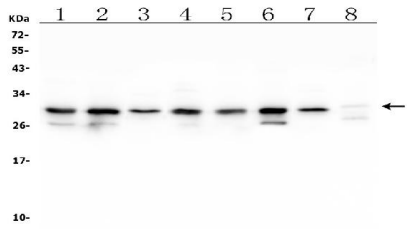


Figure 1. Western blot analysis of COMT using anti-COMT antibody (M00464).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human HepG2 whole cell lysates
Lane 2: human K562 whole cell lysates
Lane 3: human THP-1 whole cell lysates
Lane 4: human HEK293 whole cell lysates
Lane 5: human A549 whole cell lysates
Lane 6: human Caco-2 whole cell lysates
Lane 7: rat RH35 whole cell lysates
Lane 8: mouse Neuro-2a 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 mouse anti-COMT antigen affinity purified monoclonal antibody (Catalog # M00464) 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 COMT at approximately 29KD. The expected band size for COMT is at 29KD.

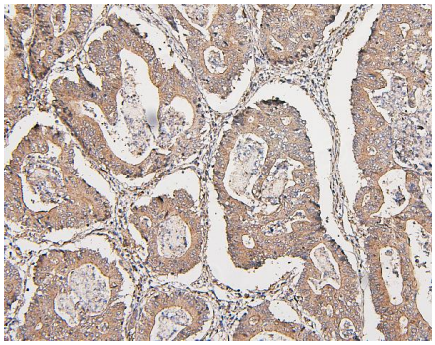


Figure 2. IHC analysis of COMT using anti-COMT antibody (M00464).
COMT was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-COMT Antibody (M00464) 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.

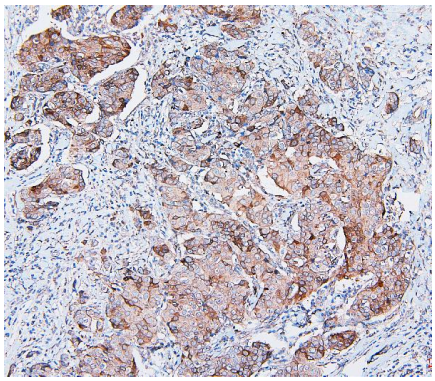


Figure 3. IHC analysis of COMT using anti-COMT antibody (M00464).
COMT was detected in paraffin-embedded section of human mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-COMT Antibody (M00464) 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.

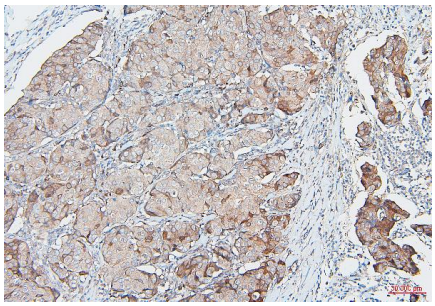


Figure 4. IHC analysis of COMT using anti-COMT antibody (M00464).
COMT was detected in paraffin-embedded section of human mammary cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-COMT Antibody (M00464) 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.

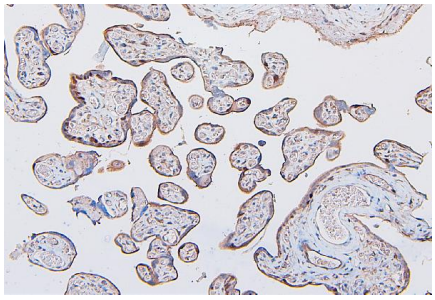


Figure 5. IHC analysis of COMT using anti-COMT antibody (M00464).
COMT was detected in paraffin-embedded section of human placenta tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-COMT Antibody (M00464) 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.

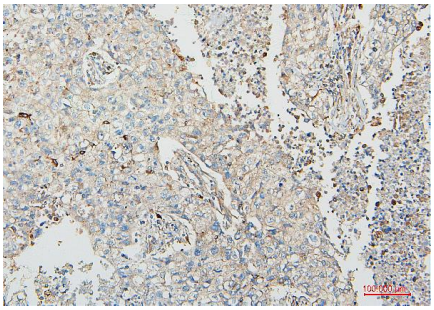


Figure 6. IHC analysis of COMT using anti-COMT antibody (M00464).
COMT was detected in paraffin-embedded section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-COMT Antibody (M00464) 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.

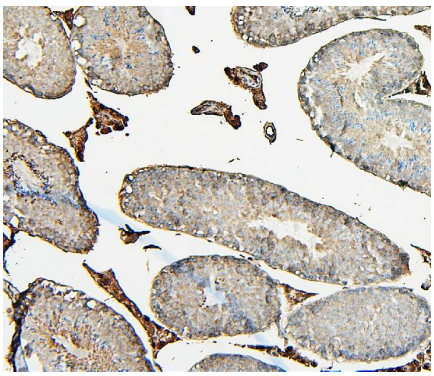


Figure 7. IHC analysis of COMT using anti-COMT antibody (M00464).
COMT was detected in paraffin-embedded section of mouse testis tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-COMT Antibody (M00464) 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.

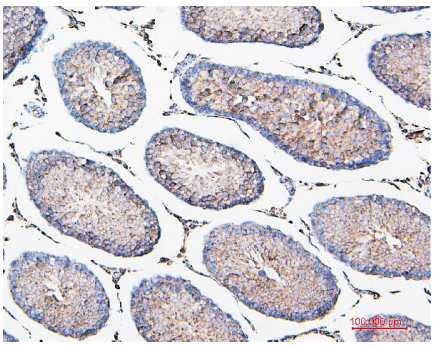


Figure 8. IHC analysis of COMT using anti-COMT antibody (M00464).
COMT was detected in paraffin-embedded section of rat testis tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-COMT Antibody (M00464) 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.

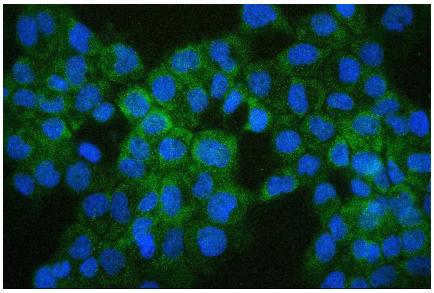
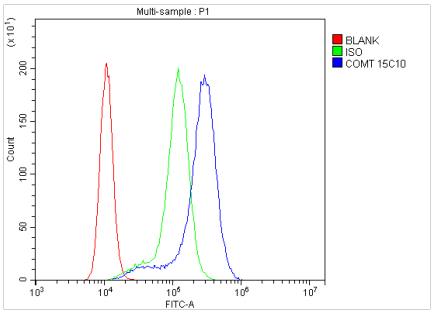


Figure 9. IF analysis of COMT using anti-COMT antibody (M00464).
COMT was detected in immunocytochemical section of A431 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-COMT Antibody (M00464) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) 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 K562 cells using anti-COMT antibody (M00464).
Overlay histogram showing K562 cells stained with



M00464 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-COMT Antibody (M00464,1 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.

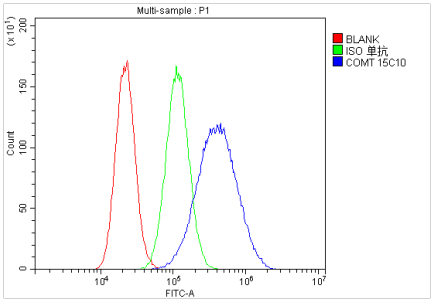


Figure 11. Flow Cytometry analysis of U87 cells using anti-COMT antibody (M00464).
Overlay histogram showing U87 cells stained with M00464 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-COMT Antibody (M00464,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 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.

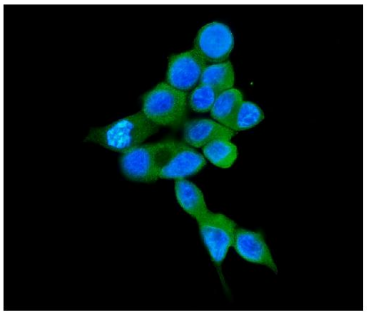


Figure 12. IF analysis of COMT using anti-COMT antibody (M00464).
COMT was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-COMT Antibody (M00464) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) 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.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.