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Anti-Transketolase/TKT Picoband™ Antibody (monoclonal, 3E5)

Catalog # ABO15005

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P29401
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Transketolase/TKT Picoband™ Antibody (monoclonal, 3E5) . 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 500ug/ml.

Additional Information

Gene ID	7086
Other Names	Transketolase, TK, 2.2.1.1, TKT
Calculated MW	67878
Application Details	Western blot, 0.1-0.25 □g/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 1-2 □g/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence; 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 3E5
Immunogen	E.coli-derived human Transketolase/TKT recombinant protein (Position: M1-A116).
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.

Protein Information

Name TKT

Function Catalyzes the transfer of a two-carbon ketol group from a ketose donor to an aldose acceptor, via a covalent intermediate with the cofactor thiamine pyrophosphate (PubMed:20667822, PubMed:27259054, PubMed:31534226). Thus, catalyzes the reversible transfer of a two- carbon ketol group from sedoheptulose-7-phosphate to glyceraldehyde-3- phosphate, producing xylulose-5-phosphate and ribose-5-phosphate (PubMed:20667822, PubMed:27259054, PubMed:31534226). The phosphate group of the substrate plays a role in stabilizing the substrate- thiamine intermediate while preventing the formation of the reactive enamine intermediate (Ref.28). Can also use fructose-6-phosphate as a ketose donor (Probable).

Background

Transketolase is a thiamine-dependent enzyme that links the pentose phosphate pathway with the glycolytic pathway. The pentose phosphate pathway, which is active in most tissues, provides sugar phosphates for intermediary biosynthesis, especially nucleotide metabolism, and generates the biosynthetic reducing power for the cell in the form of NADPH. Transketolase is directly involved in the branch of the pathway that channels excess sugar phosphates to glycolysis, enabling the production of NADPH to be maintained under different metabolic conditions. NADPH is critical for maintaining cerebral glutathione, and thus it is likely that transketolase plays an important role in brain metabolism.

Images

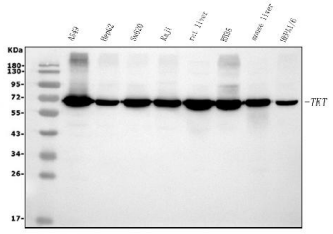


Figure 1. Western blot analysis of Transketolase/TKT using anti-Transketolase/TKT antibody (M02197). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human A549 whole cell lysates,
Lane 2: human HEPG2 whole cell lysates,
Lane 3: human SW620 whole cell lysates,
Lane 4: human Raji whole cell lysates,
Lane 5: rat liver tissue lysates,
Lane 6: rat RH35 whole cell lysates,
Lane 7: mouse liver tissue lysates,
Lane 8: mouse HEPA1-6 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-Transketolase/TKT antigen affinity purified monoclonal antibody (Catalog # M02197) 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 Transketolase/TKT at approximately 68KD. The expected band size for

Transketolase/TKT is at 68KD.

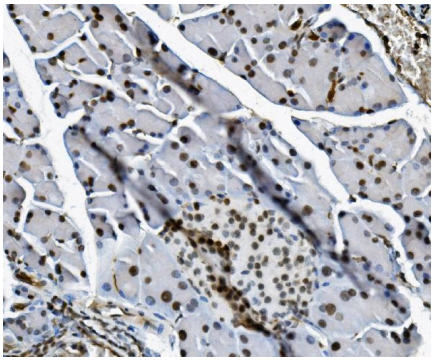


Figure 2. IHC analysis of Transketolase/TKT using anti-Transketolase/TKT antibody (M02197). Transketolase/TKT was detected in paraffin-embedded section of mouse pancreas tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Transketolase/TKT Antibody (M02197) 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.

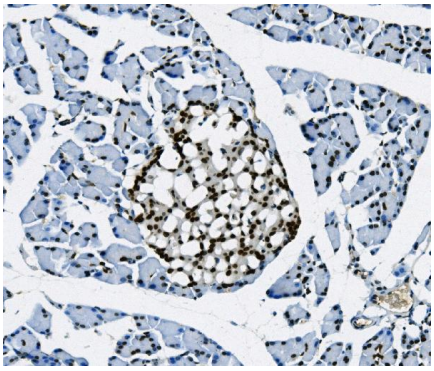


Figure 3. IHC analysis of Transketolase/TKT using anti-Transketolase/TKT antibody (M02197). Transketolase/TKT was detected in paraffin-embedded section of rat pancreas tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Transketolase/TKT Antibody (M02197) 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.

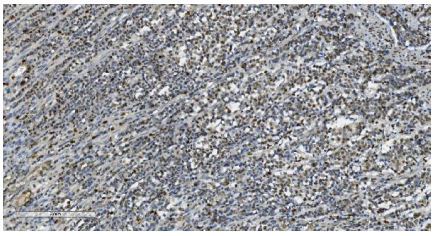


Figure 4. IHC analysis of Transketolase/TKT using anti-Transketolase/TKT antibody (M02197). Transketolase/TKT was detected in paraffin-embedded section of human gastric cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Transketolase/TKT Antibody (M02197) 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.

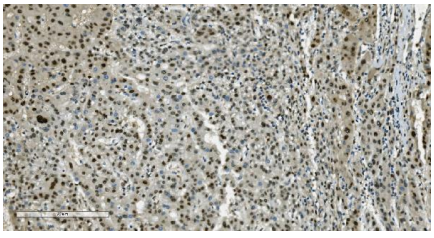


Figure 5. IHC analysis of Transketolase/TKT using anti-Transketolase/TKT antibody (M02197). Transketolase/TKT was detected in paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Transketolase/TKT Antibody (M02197) 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.

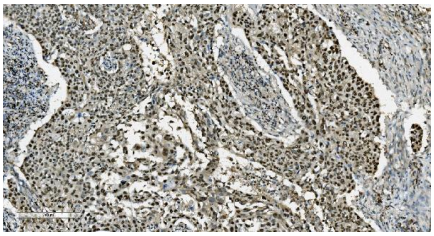


Figure 6. IHC analysis of Transketolase/TKT using anti-Transketolase/TKT antibody (M02197). Transketolase/TKT was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Transketolase/TKT Antibody (M02197) 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.

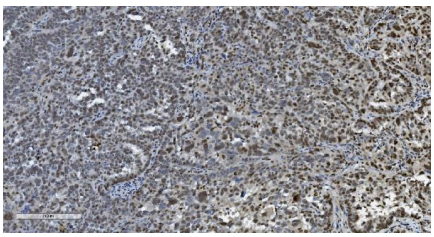


Figure 7. IHC analysis of Transketolase/TKT using anti-Transketolase/TKT antibody (M02197). Transketolase/TKT was detected in paraffin-embedded section of human ovarian cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Transketolase/TKT Antibody (M02197) 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.

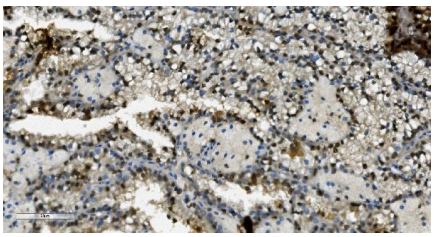


Figure 8. IHC analysis of Transketolase/TKT using anti-Transketolase/TKT antibody (M02197). Transketolase/TKT was detected in paraffin-embedded section of human renal carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Transketolase/TKT Antibody (M02197) 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.

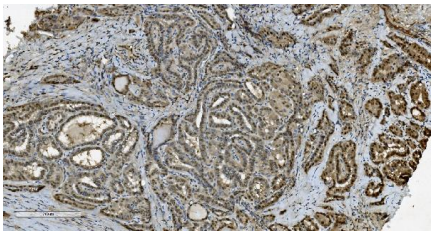
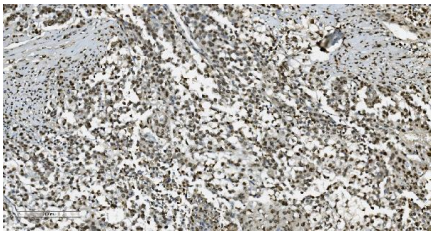


Figure 9. IHC analysis of Transketolase/TKT using anti-Transketolase/TKT antibody (M02197). Transketolase/TKT was detected in paraffin-embedded section of human thyroid cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-Transketolase/TKT Antibody (M02197) 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 10. IHC analysis of Transketolase/TKT using anti-Transketolase/TKT antibody (M02197). Transketolase/TKT was detected in paraffin-embedded



section of human pancreatic 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-Transketolase/TKT Antibody (M02197) 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.

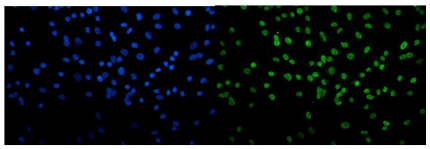


Figure 11. IF analysis of Transketolase/TKT using anti-Transketolase/TKT antibody (M02197). Transketolase/TKT was detected in immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-Transketolase/TKT Antibody (M02197) 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.

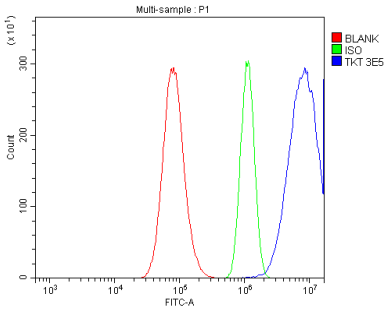


Figure 12. Flow Cytometry analysis of A549 cells using anti-Transketolase/TKT antibody (M02197).
Overlay histogram showing A549 cells stained with M02197 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Transketolase/TKT Antibody (M02197, 1 µg/1x10^6 cells) 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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