Anti-OGT Antibody [JB44-39] - BSA and Azide free

HA750459

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 117 kDa
Clone number:	JB44-39

Description: O-linked N-acetylglucosamine (O-GlcNAc) transferase (also designated OGT) catalyzes the addition of a single N-acetylglucosamine in O-glycosidic linkage to serine or threonine residues. Since both phosphorylation and glycosylation compete for similar serine or threonine residues, the two processes may compete for sites, or they may alter the substrate specificity of nearby sites by steric or electrostatic effects. O-GlcNAc transferase has been purified from rat liver. It exists as a heterotrimeric complex with two subunits of the same molecular mass and one shorter subunit. Both polypeptides are related; the short subunit band is either a proteolytic product of the polypeptide or the product of an alternative translation start site. O-GlcNAc transferase is expressed as multiple transcripts that are present in different amounts in various human tissues, with the highest levels of expression in pancreas. Immunofluorescence of human cells expressing rat O-GlcNAc transferase indicated that it is present in both the nucleus and cytosol. HeLa cells expressing O-GlcNAc transferase do not survive well during prolonged incubations, suggesting that this protein may be toxic to the cells.

Immunogen: Synthetic peptide within Human OGT aa 997-1,046 / 1,046.

Positive control: HeLa cell lysate, MCF7 cell lysate, HEK-293 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, HeLa, MCF7, PC-12, human testis tissue, human liver tissue, mouse testis tissue, mouse epididymis tissue, rat testis tissue.

Subcellular location: Nucleus. Cytoplasm. Membrane.

Database links: SwissProt: O15294 Human | Q8CGY8 Mouse | P56558 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
IHC-P	1:200-1:1,000
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

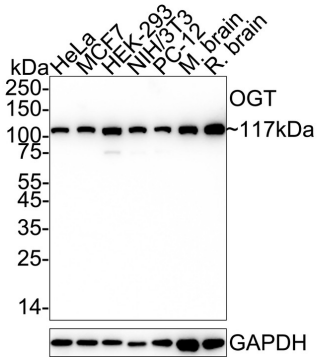

Fig1: Western blot analysis of OGT on different lysates with Rabbit anti-OGT antibody (HA750459) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: MCF7 cell lysate (20 µg/Lane)
Lane 3: HEK-293 cell lysate (20 µg/Lane)
Lane 4: NIH/3T3 cell lysate (20 µg/Lane)
Lane 5: PC-12 cell lysate (20 µg/Lane)
Lane 6: Mouse brain tissue lysate (40 µg/Lane)
Lane 7: Rat brain tissue lysate (40 µg/Lane)

Predicted band size: 117 kDa
Observed band size: 117kDa

Exposure time: 46 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750459) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

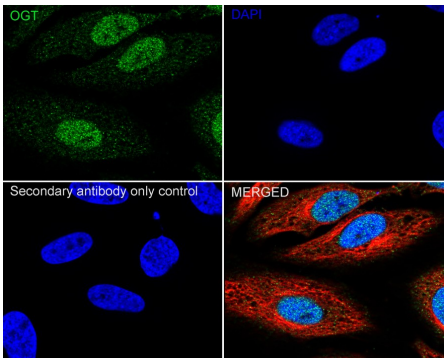

Fig2: Immunocytochemistry analysis of HeLa cells labeling OGT with Rabbit anti-OGT antibody (HA750459) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-OGT antibody (HA750459) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

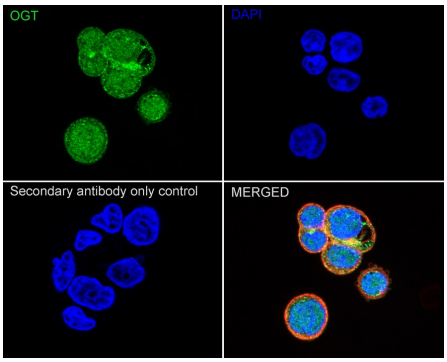

Fig3: Immunocytochemistry analysis of MCF7 cells labeling OGT with Rabbit anti-OGT antibody (HA750459) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-OGT antibody (HA750459) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

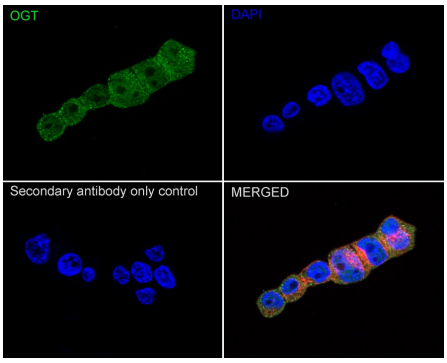

Fig4: Immunocytochemistry analysis of PC-12 cells labeling OGT with Rabbit anti-OGT antibody (HA750459) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-OGT antibody (HA750459) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

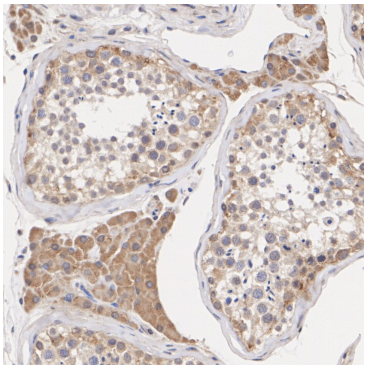

Fig5: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-OGT antibody (HA750459) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750459) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

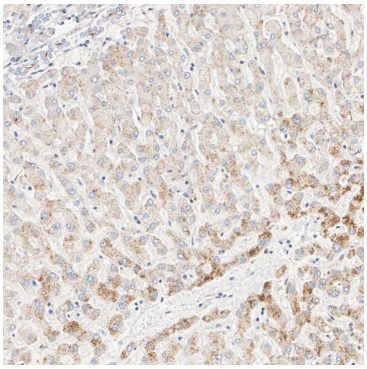

Fig6: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-OGT antibody (HA750459) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750459) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

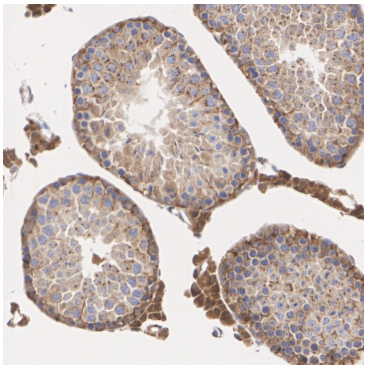

Fig7: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-OGT antibody (HA750459) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750459) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

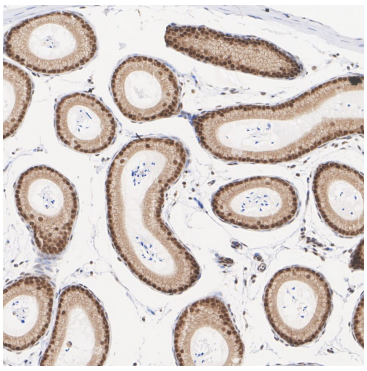

Fig8: Immunohistochemical analysis of paraffin-embedded mouse epididymis tissue with Rabbit anti-OGT antibody (HA750459) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750459) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

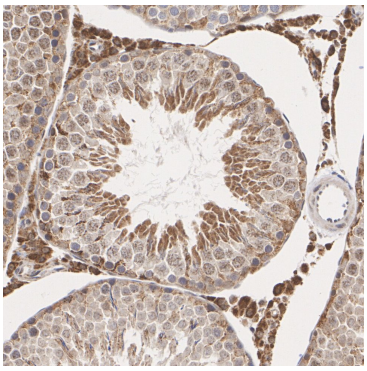

Fig9: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Rabbit anti-OGT antibody (HA750459) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750459) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

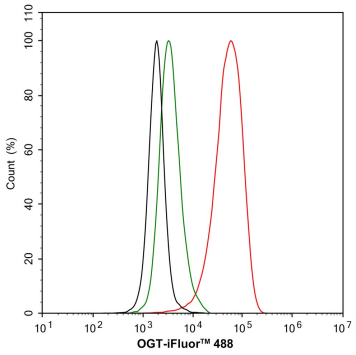

Fig10: Flow cytometric analysis of HeLa cells labeling OGT.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750459, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

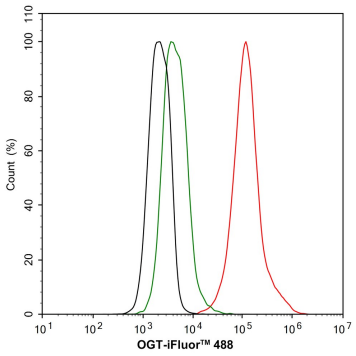

Fig11: Flow cytometric analysis of MCF7 cells labeling OGT.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750459, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

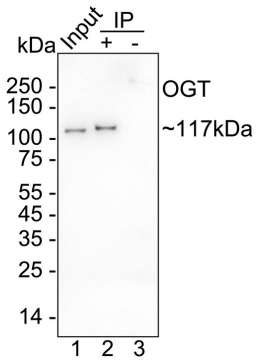

Fig12: OGT was immunoprecipitated from 0.2 mg HeLa cell lysate with HA750459 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA750459 at 1/1,000 dilution. Mouse Anti-Rabbit IgG kappa light chain secondary antibody (M1208-2) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA750459 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA750459 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 59 seconds; ECL: K1801

Background References

1. Yang X et al. Recruitment of O-GlcNAc transferase to promoters by corepressor mSin3A: coupling protein O-GlcNAcylation to transcriptional repression. Cell 110:69-80 (2002).
2. Yang X et al. Phosphoinositide signalling links O-GlcNAc transferase to insulin resistance. Nature 451:964-969 (2008).