Human Polypeptide GalNac Transferase 3/GALNT3 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG

Catalog Number: AF7174

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Polypeptide GalNac Transferase 3/GALNT3 in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant human (rh) GALNT1 and rhGALNT4 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Polypeptide GalNac Transferase 3/GALNT3 Gln38-Asp633 Accession # Q14435
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS.

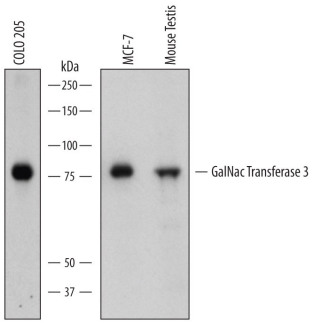
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below

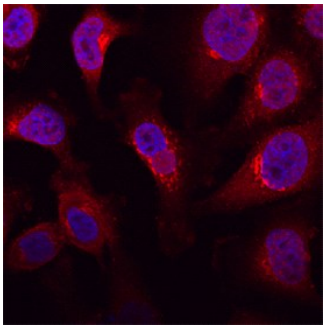
DATA

Western Blot



Detection of Human and Mouse Polypeptide GalNac Transferase 3/GALNT3 by Western Blot.
Western blot shows lysates of COLO 205 human colorectal adenocarcinoma cell line, MCF-7 human breast cancer cell line, and mouse testis tissue. PVDF membrane was probed with 0.5 µg/mL of Sheep Anti-Human Polypeptide GalNac Transferase 3/GALNT3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7174) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Polypeptide GalNac Transferase 3/GALNT3 at approximately 75 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



Polypeptide GalNac Transferase 3/GALNT3 in HeLa Human Cell Line.
Polypeptide GalNac Transferase 3/GALNT3 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Sheep Anti-Human Polypeptide GalNac Transferase 3/GALNT3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7174) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to Golgi granules. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

bio-techne
bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956

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BACKGROUND

O-glycosylation is a ubiquitous post-translational modification present in secreted and membrane-bound proteins. Polypeptide N-acetylgalactosaminyltransferases (GALNTs) calalyze the initial step for O-glycosylation by transferring GalNAc to Thr or Ser residues (GalNAc α1-O-Ser/Thr) in the Golgi compartment. Structurally, the GALNTs consist of an N-terminal catalytic domain tethered by a short linker to a C-terminal ricin-like lectin domain containing three potential carbohydrate-binding sites (1, 2). Twenty distinct GALNT isoforms have been detected in humans. These isoforms display both unique and overlapping substrate specificities (3, 4, 5) with no known universal consensus glycosylation sequence. Glycosylation of mucins results from the successive, often hierarchical, action of several specific GALNTs (6). Expression of GALNT3 appears to be highly regulated and mainly found in pancreas and testis (7). Using a peptide library screening approach, GALNT3 was classified as an intermediate transferase that increases the density of O-linked glycans within the mucin domain following glycosylation with early transferases (5). The enzymatic activity of recombinant human GALNT3 was determined using a phosphatase-coupled assay (8).

References:

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bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956