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Glycopeptide specific monoclonal antibodies suggest new roles for O-GlcNAc

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Abstract

Studies of post-translational modification by β -*N*-acetyl-D-glucosamine (*O*-GlcNAc) are hampered by a lack of efficient tools such as *O*-GlcNAc specific antibodies that can be employed for detection, isolation, and site localization. We have obtained a large panel of *O*-GlcNAc-specific IgG MAbs having a broad spectrum of binding partners by combining three-component immunogen methodology with hybridoma technology. Immunoprecipitation followed by large-scale shotgun proteomics led to the identification of more than 200 mammalian *O*-GlcNAc modified proteins, including a large number of novel glycoproteins. A substantial number of the glycoproteins were only enriched by one of the antibodies and this observation combined with results of inhibition ELISAs suggests that the antibodies in addition to their *O*-GlcNAc-dependence also appear to have different, but overlapping, local peptide determinants. The MAbs made it possible to delineate differentially modified proteins of liver in response to trauma-hemorrhage and resuscitation in a rat model.

Keywords

O-GlcNAc; Immunogen; Glycopeptide; Proteomics; Post-translational Modification

INTRODUCTION

O-glycosylation of serine and threonine of nuclear and cytoplasmic proteins by a single β -*N*-acetyl-D-glucosamine moiety (*O*-GlcNAc) is a ubiquitous post-translational modification

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AUTHOR CONTRIBUTIONS SI and GAE performed the chemical synthesis; MAW performed, analyzed and directed the immunological experiments; CFT performed and analyzed the western blotting and MS experiments; LGN and JCC were responsible for the rat model; LW and GJB were responsible for the overall experimental design and wrote the paper; and GJB was responsible for compound design.

Note: Supplementary information is available.

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that is highly dynamic and fluctuates in response to cellular stimuli through the action of the cycling enzymes, *O*-linked GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA)¹. This type of glycosylation has been implicated in many cellular processes, frequently via interplay with phosphorylation^{2,3}. Alteration of *O*-GlcNAc levels have been linked to the etiology of prevalent human diseases including type II diabetes and Alzheimer's disease⁴⁻⁷. Furthermore, a number of recent studies have shown that acute increases in *O*-GlcNAc levels attenuate cellular injury arising from a variety of stress stimuli including hypoxia and ischemia/reperfusion⁸⁻¹⁰.

Unlike phosphorylation for which a wide range of pan- and site-specific phospho-antibodies are available, studies of *O*-GlcNAc modification are hampered by a lack of effective tools for its detection, quantification and site localization. While metabolic, lectin-based and chemoenzymatic approaches have been fruitful^{11,12}, only two pan-*O*-GlcNAc specific antibodies have been described: an IgM pan-*O*-GlcNAc antibody (CTD 110.6¹³) and an IgG antibody raised against *O*-GlcNAc modified components of the nuclear pore (RL-2,¹⁴) that shows restricted cross-reactivity with *O*-GlcNAc modified proteins. Both antibodies suffer from relatively low binding affinities, and thus in many cases can only be employed for the detection of multiple modified, higher molecular weight or highly abundant proteins¹². Furthermore, IgM antibodies cannot easily be used for immunoprecipitation because they do not bind to Protein A/G-agarose and immobilization by chemical crosslinking often leads to loss of binding activity.

We reasoned that *O*-GlcNAc-specific antibodies can be elicited by employing a three-component immunogen (compound **1**, Fig. 1) composed of an *O*-GlcNAc containing peptide, which in this study is derived from casein kinase II (CKII) α subunit¹⁵, a well-documented murine MHC class II restricted helper T-cell epitope and a Toll-like receptor-2 (TLR2) agonist as an in-built adjuvant. Such a compound was expected to circumvent immune suppression caused by a carrier protein or linker region of a classical conjugate vaccine; yet it contains all mediators required for eliciting a strong and relevant IgG immune response¹⁶. It has been found that immunization with **1** followed by standard hybridoma technology gave a large panel of IgG MAbs that in addition to *O*-GlcNAc-dependence also appear to have different, but overlapping, local peptide determinants. The power of the new MAbs has been demonstrated by large-scale enrichment of *O*-GlcNAc modified proteins followed by shotgun proteomics, which led to the identification of more than 200 mammalian *O*-GlcNAc modified proteins, including a large number of novel glycoproteins. The new methodology has been applied to the identification of specific proteins that exhibit changes in *O*-GlcNAc status in response to trauma-hemorrhage and resuscitation in a rat model. We postulate that several of these proteins have the potential to modulate the response of the liver to stress.

RESULTS

Compound **1**, which was readily obtained by liposome-mediated native chemical ligation¹⁶ (Fig. 1 and Supplementary Fig. 1 online), was incorporated into phospholipid-based small unilamellar vesicles and then employed for immunization of female BALB/c mice at biweekly intervals four times (**Supplementary Methods** online). ELISA demonstrated that compound **1** had elicited excellent titers of IgG antibodies against CGSTPVS(β -*O*-GlcNAc)SANM (**3**) linked to BSA (Supplementary Table 1 online). Interestingly, immunizations with compound **2**, which has an artificial thio-linked GlcNAc moiety, did not elicit substantial higher titers of glycopeptide selective antibodies indicating that compound **1** is sufficiently metabolic stable to induce such antibodies. Furthermore, a conjugate composed of glycopeptide **3** linked to the carrier protein KLH did not elicit relevant antibodies and the immune response was directed to the carrier protein and linker.

Spleens of two mice immunized with **1** were harvested and standard hybridoma culture technology gave seven IgG1, seven IgG2a, two IgG2b and fourteen IgG3 producing hybridoma cell lines (Supplementary Fig. 2 and Supplementary Table 2 online). All MABs recognized CGSTPVS(β -*O*-GlcNAc)SANM (**3**) linked to BSA whereas only a small number bound to the peptide CGSTPVSSANM (**4**) conjugated to BSA. Furthermore, the interaction of twenty MABs could be inhibited with the glycopeptide GSTPVS(β -*O*-GlcNAc)SANM (**5**), but not with peptide GSTPVSSANM (**6**) or β -*O*-GlcNAc-Ser (**7**) demonstrating glycopeptide specificity.

Three hybridomas (18B10.C7(3), 9D1.E4(10) and 1F5.D6(14)) were cultured at a one-liter scale and the resulting IgG antibodies purified by saturated ammonium sulfate precipitation followed by Protein G chromatography. Inhibition ELISA confirmed that the MABs require carbohydrate and peptide (glycopeptide) and demonstrated that they bind subtle different epitopes. 1F5.D6(14) could be inhibited by a glycosylated tripeptide **9**, whereas 18B10.C7(3) required a glycosylated pentapeptide **8** and 9D1.E3(10) was inhibited only by the full-length B-epitope **5** (Supplementary Fig. 3 online).

To establish the usefulness of the MABs for immuno-detection, CKII α subunit was immunoprecipitated from HEK293T lysates with or without exogenous overexpression of OGT and the eluates were subjected to standard immunoblotting procedures. While equal amounts of CKII α subunit were pulled down, the monoclonal antibodies showed cross-reactivity towards a band corresponding to CKII α subunit with an increased signal for the OGT overexpressed sample supporting GlcNAc-dependence of recognition (Fig. 2a). The specificity of the MABs was further evaluated in mammalian cell crude extracts by genetically manipulating OGA or OGT levels. Importantly, three distinct global *O*-GlcNAc levels were observed, in which lysates with OGA, mock and OGT transfection yielded lowest, median and highest modification status respectively, which is in agreement with the expression levels of the cycling enzymes (Fig. 2b). The results imply that although the epitope was derived from a single protein, the MABs have a broad spectrum of binding targets. We also demonstrated that a significant number of the glycoproteins enriched by the new MABs cross react with the anti-*O*-GlcNAc IgM CTD110.6 (Fig. 2c).

The new MABs and CTD110.6 were employed for large-scale enrichment of *O*-GlcNAc modified proteins for shotgun proteomics. Agarose covalently conjugated MABs were mixed with nucleocytoplasmic proteins extracted from HEK293T cells cultured in the presence of the OGA inhibitor, PUGNAc¹⁷. The released proteins were digested by Lys-C and the recovered peptides were detected by LC-MS/MS on an LTQ-XL and analyzed by TurboSequest and ProteoIQ. Using the three new MABs, 215 *O*-GlcNAc modified proteins were identified, 140 of which are novel (**Supplementary Tables 3, 4 and 5** online). A large number of previously characterized *O*-GlcNAc modified proteins¹⁸, such as SP1, OGT and nuclear pore protein p62 were found adding confidence to proper assignment and further supports the selectivity of the antibodies for *O*-GlcNAc modification. However, the possibility that some proteins may have been co-purified due to tight association to *O*-GlcNAc modified proteins cannot be excluded. Immunoprecipitation with the IgM antibody CTD110.6 led to the identification of a limited number of proteins, highlighting the favorable properties of the new antibodies.

The extensive list of *O*-GlcNAc modified proteins made it possible to assign biological functions using HPRD (Fig. 2d). A large number of identified proteins are involved in transcriptional/translational regulation and signal transduction (Table 1, **Supplementary Tables 4 and 5**), which is consistent with recent reports that functionally implicates *O*-GlcNAc modification on insulin signaling and transcriptional control¹⁹⁻²¹. Of particular interest is that several of the glycoproteins are involved in the ubiquitin pathway. A role for

O-GlcNAc has already been established for regulation of the proteasome²² but our data indicate that *O*-GlcNAc may also be actively involved in earlier steps of the degradation cascade. SEC23 components and interacting proteins were also captured by multiple antibodies suggesting a possible role for *O*-GlcNAc modification in anterograde trafficking of intracellular vesicles. Finally, several ribosomal proteins were observed, which is in agreement with the recent finding that *O*-GlcNAc modification of ribosomal proteins plays a role in stress granule and processing body assembly²³.

Several newly identified *O*-GlcNAc proteins were observed by only one of the new antibodies (**Supplementary Tables 4 and 5**) and 1F5.D6(14) recognized the most proteins, which is consistent with its broadest inhibition profile (Supplementary Fig. 3 online). Many of the novel proteins participate in other types of post-translational modifications such as WNK2 and WNK3 for phosphorylation and RanBP2 and SUMO4 for SUMOylation. Also, a range of proteins that modulate gene expression at the chromatin levels such as SMARCC1 and CARM1 were isolated by only a subset of the new antibodies.

Next, the methodology was employed for characterization of *O*-GlcNAc modified proteins in liver samples from rats subjected to trauma-hemorrhage followed by resuscitation (TH-R) and Sham controls. Recent studies^{7-10,24} have demonstrated that loss of *O*-GlcNAcylation following TH-R leads to organ dysfunction, increased injury and decreased survival; however, no *O*-GlcNAc proteins have been identified, as potential candidates for mediating this response. As expected, evaluation of crude tissue samples by western blotting showed significantly lower overall hepatic *O*-GlcNAc levels 24 h after trauma-hemorrhage and resuscitation compared to Sham controls by all three MAb generated in this study, as well as CTD110.6 (Fig. 3 and (Supplementary Fig. 4 online)). To provide insight into proteins whose *O*-GlcNAc status is modified by trauma-hemorrhage and resuscitation (TH-R), proteins from liver extracts Sham or TH-R treated were enriched using MAb 1F5.D6(14), and analyzed by triplicate shotgun proteomics. Following stringent filtering, 68 proteins were assigned as putatively *O*-GlcNAc modified in the Sham group and 30 in the TH-R liver samples resulting in 46 novel *O*-GlcNAc modified proteins (Fig. 3 and **Supplementary Tables 6 and 7** online). While global *O*-GlcNAc levels were reduced in the TH-R samples compared to Sham, 10 of the 30 identified proteins in the TH-R samples were not detected in samples from the Sham although there appeared to be no significant changes in selected proteins abundances in crude extracts from the two groups (Supplementary Fig. 5 online).

DISCUSSION

High affinity pan-specific antibodies are expected to offer convenient and robust tools for exploring the *O*-GlcNAc proteome. However, such antibodies have been difficult to generate probably due to the fact that *O*-GlcNAc modified epitopes are self-antigens, which are tolerated by the immune system and because carbohydrates-protein interaction are relatively weak complicating antibody maturation¹². Indeed, we found that a conjugate composed of a glycopeptide derived from CKII linked to the carrier protein KLH was unable to elicit relevant antibodies and in this case the immune response was almost entirely directed to the carrier protein and linker. However, we have been able to obtain a large panel of *O*-GlcNAc-specific IgG MAbs having a broad spectrum of binding partners by combining three-component immunogen methodology with hybridoma technology¹⁶. The power of the new MAbs has been demonstrated by identifying more than 200 mammalian *O*-GlcNAc modified proteins, including a large number of novel glycoproteins. Interestingly, while there was some overlap in the assigned proteins for each antibody enrichment, a substantial number of proteins were only enriched by one of the antibodies. This observation combined with results of inhibition ELISAs using glycopeptides of

increasing complexity suggest that the antibodies in addition to their *O*-GlcNAc-dependence also have different, but overlapping, local peptide determinants.

It is to be expected that mapping of glycosylation sites of proteins immuno-precipitated with the antibodies will provide additional information about the epitope requirements of the antibodies. Although ETD was employed in this study, which is a mass spectrometric approach suitable for the detecting of glycopeptides²⁵, such structures were not found. This difficulty is probably due to the fact that glycopeptides are difficult to ionize in the presence of peptides. It is to be expected that enrichment of *O*-GlcNAc proteins using the new antibodies combined with chemoenzymatic approaches for glycopeptide isolation^{26,27} and cutting edge mass spectrometry approaches²⁸ will facilitate the site mapping of *O*-GlcNAc modified proteins.

A number of recent studies have demonstrated that acute augmentation of *O*-GlcNAc levels is associated with increased tolerance of cells to stress and conversely, inhibition of *O*-GlcNAc formation decreases cell survival^{23,24,29-32}. In a rat model of trauma-hemorrhage, increasing *O*-GlcNAc synthesis with glucosamine or inhibiting *O*-GlcNAc degradation with PUGNAc during resuscitation leads to improved organ function, decreased tissue injury, reduced inflammatory responses and lower mortality²⁴. Surprisingly, however, it was found that resuscitation resulted in marked loss of overall *O*-GlcNAc levels in multiple tissues, which was sustained for up to 24 h and that treatment with either glucosamine or PUGNAc (10) prevented this loss. Moreover, there was a strong correlation between the level of *O*-GlcNAcylation in the liver and markers of liver injury 24 h after TH and resuscitation. However, to date, identification of specific proteins that exhibit changes in *O*-GlcNAc modification in response to trauma-hemorrhage and resuscitation has been difficult. Here, we have employed the new antibodies to identify a number of novel *O*-GlcNAc proteins targets, which potentially can modulate the response of the liver to stress.

A relatively large number of identified proteins is involved in metabolism, which is in agreement with the fact that the liver is a metabolically active organ. The metabolic proteins include those related to lipogenesis, including glycerol-3-phosphate dehydrogenase, ATP citrate lyase and malic enzymes, gluconeogenesis, such as fructose-1,6-biphosphatase, and lipid oxidation, such as acyl-CoA dehydrogenase and acetyl-Co acyl transferase. Fructose-1,6-biphosphatase is of particular interest since it provides an alternative to glucose-6-phosphate isomerase for the generation of fructose-6-phosphate, which is the primary substrate for the hexosamine biosynthetic pathway and the synthesis of UDP-GlcNAc, the donor sugar nucleotide for *O*-GlcNAc modification. Furthermore, recent evidence suggests that transcription of gluconeogenic enzymes are regulated by *O*-GlcNAc modification^{20,33}. The intriguing possibility of *O*-GlcNAc regulating gluconeogenic enzymes directly in addition to regulating their transcription awaits future studies. Following TH-R there was a marked decrease in the total number of *O*-GlcNAc modified proteins identified compared to the Sham group, which is consistent with the fact that the overall *O*-GlcNAc levels are reduced upon TH-R. However, several proteins identified in the TH-R samples were not detected in the Sham, which is in agreement with the recent finding that changes in *O*-GlcNAc modification can occur on a subset of modified proteins in response to a stimuli without altering *O*-GlcNAc levels on other proteins²⁶.

A number of proteins related to oxidative stress including superoxide dismutase, thioredoxin and glutathione *S*-transferase were identified providing a possible rationale for the fact that increased overall *O*-GlcNAc levels protect against oxidative stress. Identification of these glycoproteins also lends support for a potential crosstalk between redox and *O*-GlcNAc signaling pathways⁷.

A subset of ten proteins was *O*-GlcNAcylated only in the TH-R group. One of these proteins Death associated protein (DAP)-kinase 3 is a Ca^{2+} /calmodulin (CaM)-regulated Ser/Thr kinase, which is a positive mediator of programmed cell death³⁴. Autophosphorylation of DAP-kinases is believed to restrain its proapoptotic activity, whereas dephosphorylation and subsequent binding to CaM stimulates its apoptotic functions. We are unaware of any studies demonstrating a role for DAP-kinases in mediating the response of liver injury following trauma-hemorrhage and resuscitation or indicating that it is subjected to *O*-GlcNAc modification. However, it is clear that modulation of DAP-kinase activity is critical to the regulation of apoptosis and cellular homeostasis³⁴. Thus, the new tools have opened up new avenues of exploration for modulation of protein function by *O*-GlcNAc and should facilitate this future research.

METHODS

Synthetic methods

Procedures for the synthesis of (glyco)peptides, analytical data for compounds **1-9**, conjugation to BSA-MI and the preparation of liposomes are provided in the **Supplementary Methods**.

TH-R shock model

Liver samples were obtained from rats 24 h after trauma-hemorrhage and resuscitation as described in detail elsewhere³⁵. Briefly under isoflurane anesthesia soft-tissue trauma was induced by a midline laparotomy followed by hemorrhage induced by withdrawal of 55% of the calculated total blood volume. Subsequently, mean arterial pressure was maintained at 35-40 mmHg for 45 min by i.v. administration of small volumes of NaCl (0.9%). This was followed by resuscitation, with four times of total withdrawn blood volume of i.v. NaCl (0.9%), administered over 60 min. The animals were allowed recover and observed for up to 24 h after resuscitation, at which point surviving animals were euthanized by i.v. injection of concentrated KCl solution and liver collected for subsequent analysis. Sham surgery animals underwent only general anesthesia and vessel cannulation. All animals received buprenorphine (0.3 mg/Kg body wt) subcutaneously immediately following and 12 h after resuscitation. All animal experiments were approved by the University of Alabama Institutional Animal Care and Use Committee and conformed to the *Guide for the Care and Usage of Laboratory Animals*, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996).

Mass spectrometry

The dried peptide samples were resuspended in 20 μL aqueous formic acid (0.1%) / acetonitrile (2%) and filtered (0.2 μm , Nanosep, PALL). The samples were loaded off-line onto a nanospray tapered capillary column/emitter (360 $\times$ 75 $\times$ 15 μm , PicoFrit, New Objective) self-packed with C18 reverse-phase (RP) resin (8.5 cm, Waters) in a Nitrogen pressure bomb for 10 min at 1000 psi (5 μL load) and then separated *via* a 160-min linear gradient of increasing mobile at a flow rate of 200 nL/min directly into a linear ion trap mass spectrometer (LTQ-XL with ETD; ThermoFisher) as described previously³⁶. The samples derived from HEK293T were subjected to triplicate analysis using three different modes of MS/MS analysis: (1) ETD (electron transferred dissociation) mode, where a full MS spectrum was collected followed by 6 MS/MS spectra using ETD (enabled supplemental activation) of the most intense peaks. The dynamic exclusion was set at 1 for 30 sec of duration. (2) CID-NL (collision induced dissociation-pseudo neutral loss) mode, where a full MS spectrum was collected followed by 8 MS/MS spectra using CID of the most intense peaks. Upon encountering a pseudo neutral loss event (a loss of GlcNAc, 203.08), a MS^3 spectrum was generated from the resulting ion that had undergone a neutral loss. The

dynamic exclusion was the same in all methods. (3) DDNL-ETD (Data dependent neutral loss MS³ using CID followed by an ETD MS/MS spectra for any parent ion that generated a neutral loss), where MS/MS spectra from the top 5 peaks of each full MS scan were collected with CID (35% normalized collision energy) and monitored for a neutral loss of 203.08. For tryptic peptides prepared from the *O*-GlcNAc modified protein isolated from Sham or TH-R treated rat liver samples analysis was carried out in mode (2) CID-NL as described above in triplicate.

Mass spectrometry data analysis and validation

MS spectra were searched against the human or rat forward and reverse databases extracted from the appropriate species-specific Swiss-Prot proteome database using the TurboSequest algorithm (Bioworks 3.3, Thermo Finnigan). The DTA files were generated for spectra with a threshold of 15 ions and a TIC of 1e3. Dynamic mass increases of 15.99, 57.02 and 203.08 Da were considered for oxidized methionine, alkylated cysteine and *O*-GlcNAc modified serine/threonine, respectively. The resulting OUT files obtained from a search of the forward and reversed databases obtained from the different runs were combined and parsed with ProteoIQ (BioInquire, Inc.) and filtered to 1% FDR (metric used: F-value) at the protein level using a minimum for each peptide of 3% FDR. For both the human and rat data, for each individual antibody, all results were combined using ProteoIQ (BioInquire, Inc.). Proteins that appear in the filtered lists of both negative control and experimental groups were considered as contaminants and manually removed from the compiled lists.

Statistical analysis

Statistical significance between groups was determined by two-tailed, unpaired Student's *t* test. Differences were considered significant when $P < 0.05$.

Other methods

See **Supplementary Methods** for preparation of liposomes, immunization schedule, hybridoma culture and antibody production, reagents, serologic assays, plasmid construction, cell culture and transfection, immunoprecipitation, preparation of liver lysates, western blotting, sample preparation for LC-MS/MS analysis and statistical analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Structures of fully synthetic three-component immunogens **1** and **2**. Compounds **3-9** were employed for ELISA and inhibition ELISA to determine epitope selectivities of the antibodies.

Figure 2.

Immunoblotting of three monoclonal antibodies. **(a)** CKII α subunit was immunoprecipitated from HEK293T cells with or without OGT overexpression. Eluates were resolved by SDS-PAGE and immunoblotted with MAb 18B10.C7(3), 9D1.E4(10) and 1F5.D6(14). A band corresponding to CKII α subunit was detected with signal intensity correlated with *O*-GlcNAc status. All blots were stripped and reprobed with antibody against CKII α subunit (only one representative blot is shown here). Also, equal amount of CKII α subunit was present in the input regardless of the status of *O*-GlcNAc levels. **(b)** HEK293T lysates with low (OGA overexpression), median (Mock transfection) and high (OGT overexpression) levels of *O*-GlcNAc modification were exposed to MAb 18B10.C7(3),

9D1.E4(10) and 1F5.D6(14) respectively. The signals obtained mirror the corresponding *O*-GlcNAc status in each sample. Immunoblots against OGT, OGA and tubulin are also shown. While equal loading of tubulin was detected in all samples, higher OGA and OGT protein levels were detected with lysates from OGA and OGT transfections, respectively. Note: Endogenous OGT and OGA levels do appear after longer exposure. **(c)** *O*-GlcNAc proteins were immunoprecipitated from HEK293T cells treated with PUGNAc (an OGA inhibitor), resolved by SDS-PAGE and subjected to CTD110.6 (an IgM isotype *O*-GlcNAc specific antibody) blotting. Cross-reactivity of MAbs 18B10.C7(3), 9D1.E4(10) and 1F5.D6(14) with CTD110.6, albeit distinct in pattern, were detected. **(d)** Application of MAbs for *O*-GlcNAc-omics in cell culture: Distribution of *O*-GlcNAc modified proteins based on their biological process categorized in HPRD (number of individual proteins assigned to each category is displayed).

Figure 3. Application of MABs for *O*-GlcNAc-omics in mammalian tissue. **(a)** Western blot analysis using the *O*-GlcNAc antibodies of rat liver extracts following Sham or TH-R treatment. **(b)** Distribution of *O*-GlcNAc modified proteins in rat liver based on their biological process following Sham or TH-R treatment (number of individual proteins assigned to each category is displayed).

Table 1

Novel proteins enriched from HEK293T cells by more than one of the antibodies

UniProt Accession Number	Gene Name	Sequence Name	Total Peptides				Biological Process	Primary Localization	Alternate Localization
			18B10.C7 (3)	9D1.E4 (10)	1F5.D6 (14)	CTD110.6			
P49790	NUP153	Nuclear pore complex protein Nup153	3	6	30	22	Tp	N	
Q2KHR3	QSER1	Glutamine and serine-rich protein 1 (FLJ21924)	1	2	11	3	U	U	
Q02543	RPL18A	60S ribosomal protein L18a	4	3	3	3	Tl	C	No,M
P35658	NUP214	Nuclear pore complex protein Nup214	6	7	27	0	Tp	N	
Q17RM7	C11orf30	Chromosome 11 open reading frame 30 (Protein EMSY)	0	1	11	0	Gn	N	C,PM
Q8WWM7	ATX2L	Ataxin-2-like protein	2	0	10	4	U	N	C
P62753	RPS6	40S ribosomal protein S6	2	0	7	0	Tl	C	No,C
P52948	NUP98	Nuclear pore complex protein Nup98-Nup96 precursor [Contains: Nuclear pore complex protein Nup98]	0	7	6	6	Tp	N	
Q9P2N5	RBM27 (KIAA1311)	RNA-binding protein 27	0	5	6	2	U	C,N	
P78406	RAE1 (MRNP41)	mRNA export factor (mRNA-associated protein mrnp 41; Rae1 protein homolog)	0	1	6	2	Tp	N	C,M
Q12771	--	P37 AUF1	5	4	5	0	U	U	
Q8NC51	PAIRB (SERBP1)	Plasminogen activator inhibitor 1 RNA- binding protein (SERPINE1 mRNA-binding protein 1)	3	0	4	2	Gn	C	N
P26373	RPL13	60S ribosomal protein L13	2	0	4	2	Tl	C	No,N, M
P09211	GSTP1 (GST1)	Glutathione S-transferase P	5	6	4	0	Mb	C	N,M
Q15436	SEC23A	Protein transport protein Sec23A (Sec23 related protein A)	2	3	4	0	Tp	C	ER
Q99567	NUP88	Nuclear pore complex protein Nup88	0	2	4	0	Tp	N	
P11940	PABPC1 (PABP1)	Polyadenylate-binding protein 1 (Poly(A)- binding protein 1; Polyadenylate binding protein, cytoplasmic, 1)	3	1	4	0	Gn	C	N
Q14684N	RRP1B (KIAA0179)	RRP1-like protein B (Ribosomal RNA processing protein 1 homolog B)	1	0	4	0	Tl	No	N

UniProt Accession Number	Gene Name	Sequence Name	Total Peptides				Biological Process	Primary Localization	Alternate Localization
			18B10.C7 (3)	9D1.E4 (10)	1F5.D6 (14)	CTD110.6			
P83731	RPL24	60S ribosomal protein L24	2	0	3	3	Tl	C	
P16402	HIST1H1D	Histone H1.3	1	0	3	1	Gn	N	No,M
P14866	HNRNPL	Heterogeneous nuclear ribonucleoprotein L	4	0	2	2	Gn	N	No,C, M
Q15437	SEC23B	Protein transport protein Sec23B (Sec23 related protein B)	1	4	2	0	Tp	ER	G,C
Q9GZZ1	NAT13	N-acetyltransferase 13 (MAK3)	3	2	2	0	U	C	
P46779	RPL28	60S ribosomal protein L28	2	0	2	0	Tl	C	No
P05387	RPLP2	60S acidic ribosomal protein P2	2	0	2	0	Tl	C	No
Q12906	ILF3 (NFAR)	Interleukin enhancer-binding factor 3 (Nuclear factor associated with dsRNA)	4	3	1	0	Gn	N	C,No, M
Q13200	PSMD2	26S proteasome non-ATPase regulatory subunit 2	0	1	1	0	Mb	C	
P31689	DNAJ1 (HSPF4)	DnaJ homolog subfamily A member 1 (Heat shock 40 kDa protein 4)	1	0	1	0	Mb	C	N,No, G
P16403	HIST1H1C	Histone H1.2	1	0	1	0	Gn	N	No,M
Q9Y3F4	STRAP	Serine-threonine kinase receptor-associated protein	6	5	0	0	Sg	C	N
Q14011	CIRBP (A18HNRNP)	Cold-inducible RNA-binding protein (Glycine-rich RNA-binding protein; hnRNP A18)	2	2	0	0	Sg	N	
P10599	TXN	Thioredoxin	2	2	0	0	Mb	C	N,Ex
Q13151	HNRNPA0	Heterogeneous nuclear ribonucleoprotein A0	2	1	0	0	Gn	N	C,No, M
P07195	LDHB	L-lactate dehydrogenase B chain	2	1	0	0	Mb	C	
Q09028	RBBP4	Histone-binding protein RBBP4 (Retinoblastoma binding protein 4)	1	1	0	0	Gn	N	No,M

Abbreviations: Gn, Gene expression / Transcription; Mb, Metabolism; Sg, Signal transduction; St, Structural; Tl, Translation; Tp, Transport; U, Unknown; C, Cytoplasm; N, Nucleus; M, Mitochondrial; No, Nucleolus; ER, Endoplasmic reticulum; G, Golgi apparatus; Ex, Extracellular; PM, Plasma membrane.