

Develop Better Assays for Every Human Protein

OriGene Technologies, Inc.

About OriGene



- Started in1996 with the goal to create largest commercial collection of full-length human cDNAs
- Develop technologies focusing on systems biology approaches to study gene function
- Develop multiplex assays for every human protein

Largest ORF Collections



- 25,000 human and 12,000 mouse ORFs
- Including 3,000 tough genes that are only available from OriGene
- > 14,000 ready for immediate delivery



Highest Quality Standard



Not just sequence-verification



But also

Expression Validation on 12,000 TrueORF clones





APO3C TrueORF:RC206566 ORF size: 300 bp Predicted MW: 11 kd

TP53 TrueORF:RC200003 ORF size: 1182 bp Predicted MW: 53 kd



ENC1 TrueORF:RC201842 ORF size: 1770 bp Predicted MW: 65 kd



CDH3 TrueORF:RC207346 ORF size: 2490 bp Predicted MW: 91 kd



LRP1 TrueORF:RC218369 ORF size: 13635 bp Predicted MW: 504 kd

Complete Gene/clone Resource







Application of Over-expression Protein Lysates

Transfected Over-Expression Lysates



- 12,000 TrueORF clone transfected HEK293T cell lysates
- Delivered in modified RIPA buffer to preserve protein function
- Ideal tools for antibody validation, protein-protein interactions, and as assay standards



Over-expression Lysates



Phpr	the project	protein atlas	dictionary	disclaimer	submission	of antibodies help
	HUN		PROT	EIN A	TLA	AS
	The humar in a large v with the aid immunoflu	n protein atlas sho variety of normal h d of immunohistoci orescence (IF) con	teins A nes ti fi	010-03-26 new version (6.0) has been eleased including antibodies argeting protein products rom 8489 human protein- oding genes.		
	Enter seam	ch: Advanced search	<u>h</u> 9	search	N P P P	lew protein classes for otential membrane proteins ave been added, and the rotein model has been xtended with new membrane rotein topology prediction nethods.
	Or a protei	6 1 7 1 8 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	14 15 15 16 16 17 17 17 17 17 17 17 17 17 17 17 17 17	18 22 1 19 X 20 V OTHER ases Peptidases		The Human Protein Atlas now ontains subcellular ocalization data (IF) for <u>121</u> genes.

OriGene over-expression lysates adopted in Antibody Atlas Program to validate polyclonal antibodies.

Success rate is improved from 32% with regular cell lysates to 89% with over-expression lysates.

Data from Prof Mathias Uhlén, Program Director.

10,000 Lysate Protein Arrays





Protecontrols

More than 20,000 features (10,000 unique overexpression lysates) and purified control proteins were spotted on this single nitrocellulose slide. To examine the morphology for each feature, the slide was stained with colloidal gold for total protein.

10,000 Lysate Protein Arrays







Enlarged image

Anti-DDK (Rabbit)

Anti-E-Cadherin1 (mAb)

Using OriGene antigen microarray chip technology to decode antibodies that were generated by whole cell immunization

Ovarian Cancer Early Diagnostics





Figure 2: Heatmap of the proteins with high expression in at least one of the early stage tumor samples.



More than 100 patient sera were tested. Identified 200 positive hits. In collaboration with Dr. Bast in MD Anderson.



Application of Purified Proteins

Full Length Human Proteins



- Produced from TrueORF cDNA clones
- 5,000 full length human proteins
- Expressed in HEK293 cells
- Optimal preservation of protein structure, post-translational modifications and functions





mTOR Activity Data





mTOR Activity Assay

Homogeneous time-resolved fluorescent (HTRF®) assay.

mTOR is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription. mTOR belongs to the phosphatidylinositol 3kinase-related kinase protein family.

First commercial active, full-length human mTOR protein

RUNX1 Regulates MYL9





A. Koneti Rao, et al. Blood 116(26), P6037-6045, 2011

- RUNX1 mutation associated with familial platelet disorder
- MYL9 expression level and its protein MLC phosphorylation decreased in these patients
- In an EMSA experiment (electrophoretic mobility shift assay), recombinant RUNX1 binds to oligo probe (A), wt probe (B) of MYL9 promoter region
- ChIP, siRNA data also support MYL9 is a transcriptional target of RUNX1

Other publication using recombinant protein in EMSA assays to study transcription factors: 1.hnRNP k as a transactivator for human LDLR. J. Liu, JBC 285 (23), P17789-17797, 2010 2.ER regulate ATBF1 expression. J. Dong, JBC 286 (16), p13879-13890, 2011

PAGE Analysis Showing MBNL1-mediated Inclusion of IR Exon 11 in spliced mRNA



Data courtesy of the Cooper lab, Baylor College of Medicine

Protein Functional Data







Refolding Activity of HSP70





Protein Concentration (ng/ul)

MTOR (TP320457) Activity in an HTRF Assay

MS Collaboration with ISB



- Generate signature peptide sequences for all 5,000 human proteins
 - Sequences will be in PeptideAtlas database
- Heavy-isotope labeled purified proteins
 5,000 available now for SRM/MRM application
- Isotope labeled peptides

 Coming soon for all 5,000 human protein



MS Validation by ISB – AKT1





Protein Coverage = 49.3% (59.3% of likely observable sequence)

Distinct Observed Peptides (22)

(1) show column descriptions

Accession	Pre AA	Sequence	Fol AA	ESS	Best Prob	Best Adj Prob	N Obs	EOS	SSRT	N Prot Map	N Gen Loc	N S
▲ ▼	$\blacktriangle \mathbf{V}$	A V	▲ ▼				▲ ▼		A V	▲ ▼	▲ ▼	
PAp00011660	K	TFCGTPEYLAPEVLEDNDYGR	Α	1.00	1.000		2	1.00	37.24	1	0	1
PAp00354293	R	FYGAEIVSALDYLHSEK	N	1.00	1.000		2	1.00	45.04	1	0	1
PAp01459353	R	VTMNEFEYLK	L	0.99	0.998		2	1.00	30.99	1	0	1
PAp00140093	R	FFAGIVWQHVYEK	K	0.83	0.999		1	1.00	37.82	1	0	1

Heavy Labeled Proteins



- Heavy isotope labeled MAP2K1 and MAPK1 prepared by transfecting HEK293T cells with overexpression clones.
- The cells were cultured in heavy isotope medium for over 10 generations
- Proteins were isolated using anti-DDK affinity column.
- The heavy labeled proteins were sent to ISB for MS analysis and the incorporation efficiency were determined.

MRM-MS for MAP2K1





Protein: MAP2K1 (Cat#: PH318460) (

(Peptides detected by mass spec are I



Predicted m/z for +2 ion (M+2H)2+ : Light: 674.3670 Heavy: 678.3741

MS Spectra of Tryptic Digested MAP2K1





mAb for Every Human Protein







Dedicated mAb center

- Capacity: 200 immunizations per month

Authentic proteins as antigens

- Full length proteins made in human cells

Extensive validation

- WB: 9 different cell lines
- IHC: 24 different tissues
- IF
- Flow
- Luminex

Goal: produce high quality mAb to every human proteins in 5-10 years

HTP Validation of mAbs





Anti-KRT WB with OriGene over expression lysate



Anti-KRT WB with cell panel of 9 different cell lines (For endogenous protein detection in cell line)



ERCC1 (8F1)



ERCC1 (1A3)



ERCC1 (4F9)

IHC data for human carcinoma liver tissue (12 IHC data from normal human tissue, and 12 carcinoma tissue IHC tests)

IF and Flow validation of mAbs





IF data with A549 cells (For endogenous protein)



IF data with COS7 cells transiently transfected by RC clone



Flow data analysis for transfected COS7 cells (Hybridism supernatant analysis)

First LGR5 mAb for Flow Cytometry





HEK293 cells were transfected with LGR5 expression plasmid and immunostained with or without anti-LGR5 for flow cytometry test.

Immunoassay Development



Quantitation of 6 Growth Factors in an 11-plex ELISA



TrueMAB Summary



- 97% of TrueMAB antibodies work for WB application.
- More than 60% are positively validated with IF application.
- More than 50% are positively validated with IHC and Flow applications.
- For every TrueMAB antibody, an overexpression lysate positive control is included for free, and we offer moneyback guarantee.





- OriGene offers the most comprehensive proteins for functional studies and compound screening
 - 12,000 over-expression lysates
 - 5,000 purified human proteins
- Proteome wide assay technologies are coming of age. High density arrays and MRM-MS hold great promise in biomarker discovery and validation

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