

APPLICATION GUIDE

TruePLEX™ Antibody Profiling Array: Human NSC Lung Cancer III

Part Number: AP100022

For multiplex detection of human antibodies to the following proteins with the Luminex xMAP® system.

Name	Gene Symbol	Name
Cyclin A1	CCNA1	Cyclin A1
Cyclin D1	CCND1	Cyclin D1
CDK2	CDK2	Cyclin-dependent kinase 2
IMP1	IGF2BP1	Insulin-like growth factor 2 mRNA binding protein 1
Survivin	BIRC5	Baculoviral IAP repeat containing 5

For use in conjunction with the TruePLEX™ Human Antibody Profiling Kit
Part Number: AP100001

For Research Use Only

TABLE OF CONTENTS

Ordering Information	3
Custom Profiling Arrays	3
Storage Instructions	3
Overview and Intended Use	3
Assay Principles	4
Protein Source and characterization	4
Important Information	4
Recombinant Proteins.....	5
Assay Protocol.....	5
Creating larger multiplexes with additional Protein Bead Arrays	6
Troubleshooting	6
References	6

Ordering Information

TruePlex™ Human Antibody Profiling Kit (96 tests)

Catalog #: AP100001

TruePlex™ Antibody Profiling Arrays for NSC Lung Cancer:

Human NSC Lung Cancer I 5-plex (96 tests) Catalog # AP1000020	Annexin 1 (ANXA1), NY-ESO-1 (CTAG1B), p53 (TP53), c-Myc (MYC), IMPDH (IMPDH1)
Human NSC Lung Cancer II 5-plex (96 tests) Catalog # AP100021	CAGE (DDX48 / EIF4A3), HuD (ELAV4), SOX 2, MAGE A4, Annexin II (ANXA2)
Human NSC Lung Cancer III 5-plex (96 tests) Catalog # AP100022	Cyclin A1 (CCNA1), Cyclin D1 (CCND1), CDK2, IMP1(IGF2BP1), Survivin (BIRC5)
Human NSC Lung Cancer IV 5-plex (96 tests) Catalog # AP100023	alpha-enolase (ENO1), Erp 29, Fumarate hydratase (FH), HSD17B10, mortalin (HSPA9)
Human NSC Lung Cancer V 5-plex (96 tests) Catalog # AP100024	Ubiquilin (UBQLN1), p62 / IMP2 (IGF2BP2), MTAP, 3-oxoacid CoA transferase (OXCT1), Phosphoglycerate mutase (PGAM1)

Additional panels are available for breast, prostate, pancreatic, and ovarian cancer as well as for many autoimmune antigens. (see: <http://www.origene.com/Luminex/Proteinarray.aspx>)

Multiple profiling arrays can be mixed together and analyzed as a larger multiplex.

Custom Profiling Arrays

Custom profiling arrays comprising beads coupled to any of OriGene's 8400+ recombinant proteins are available. Please send an inquiry to assays@origene.com.

Storage Instructions

- Store the vial of bead mix at -20°C.

Overview and Intended Use

Human autoantibodies are known to play a pivotal role in many diseases including over 170 human autoimmune diseases and many cancers. Many autoantibody antigens have been identified, but others remain elusive or difficult to confirm. In addition, many questions remain unanswered regarding the role of autoantibodies in the pathology and natural history of the disease. OriGene has purified over 8000 recombinant human proteins from human cells that can be

used for autoantibody testing. This kit is intended to be used as a basic research tool for the detection of human autoimmune antibodies in serum and plasma for basic and clinical research studies. It is not intended for use in clinical diagnostics.

Assay Principles

Recombinant human proteins have been expressed and purified from a human cell line (HEK-293T). Purified recombinant proteins have been coupled to Luminex beads. Each protein is coupled to a different type or “color” of Luminex beads. A bead mix is prepared by combining all of the beads along with a set of control beads that are supplied with the detection kit. Diluted serum or plasma is mixed with the beads and autoantibodies, if present, will bind to the specific protein-coupled beads. After washing, an anti-human-phycoerythrin conjugate is added to detect the bound human IgG. After a final wash, the samples are read in the Luminex 100 or Luminex 200 instrument. The median fluorescent intensity measured for each bead reflects the amount of human IgG bound to the bead.

Protein Source and characterization

The recombinant proteins coupled to the Luminex beads have been expressed in human HEK-293 cells. Therefore, serum or plasma samples may react differently to these proteins than those expressed in *E. coli* or insect cells due to potential conformational differences and the presence of post-translational modifications on the proteins expressed in human cells.

The recombinant proteins are expressed with a fusion tag on the C-terminus:

Protein - TRTRPLEEQKLISEEDLAANDILDYKDDDDKV

The sequence **EQKLISEEDL** is known as the myc tag.

The sequence **DYKDDDDK** is known as the DDK or FLAG™ tag.

The tag provides for efficient purification of the recombinant proteins from the HEK-293 cells and allows for verification that sufficient protein has been coupled to the beads. The tag sequence also serves as a positive control for the assay performance.

Important Information

- **Research Use Only.** The product you have received is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic or therapeutic application. Uses other than the labeled intended use may be a violation of applicable law.
- **Hazards.** It is the end-user's responsibility to consult the applicable MSDS(s) before using this product. Disposal of waste materials must comply with all appropriate federal, state, and local regulations. If you

have any questions concerning the hazards associated with this product, please call OriGene Technologies Inc at 1-888-267-4436.

- Terms and Conditions:** By opening the packaging containing this Assay Product (which contains fluorescently labeled microsphere beads authorized by Luminex Corporation) or using this Assay Product in any manner, you are consenting and agreeing to be bound by the following terms and conditions. You are also agreeing that the following terms and conditions constitute a legally valid and binding contract that is enforceable against you. If you do not agree to all of the terms and conditions set forth below, you must promptly return this Assay Product for a full refund prior to using it in any manner. You, the customer, acquire the right under Luminex Corporation's patent rights, if any, to use this Assay Product or any portion of this Assay Product, including without limitation the microsphere beads contained herein, only with Luminex Corporation's laser based fluorescent analytical test instrumentation marketed under the name Luminex Instrument.
- Safety and Use:** All biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of potentially infectious or hazardous agents. This product is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic application. Uses other than the labeled intended use may be a violation of applicable law.

Recombinant Proteins

Each recombinant protein is coupled to a different Luminex bead. Bead assignments can be customized.

Gene Symbol	Name	OriGene Protein SKU
CCNA1	Cyclin A1	TP306615
CCND1	Cyclin D1	TP304957
CDK2	Cyclin-dependent kinase 2	TP300494
IGF2BP1	Insulin-like growth factor 2 mRNA binding protein 1	TP316226
BIRC5	Baculoviral IAP repeat containing 5	TP305935

Additional lung cancer markers may be available. Contact OriGene for an updated list. (assays@origene.com).

Assay Protocol

Refer to the protocol in the TruePLEX™ Human Autoantibody Profiling Kit

Creating larger multiplexes with additional Protein Bead Arrays

Additional protein-coupled bead mixes are available and may be combined to create larger multiplexes. Custom beads arrays can also be ordered utilizing any of OriGenes over 8000+ human-expressed purified proteins. Send an inquiry to assays@origene.com for more information.

Troubleshooting

Refer to the application guide for the TruePLEX™ Human Autoantibody Detection Kit.

References

- Chapman CJ, Murray A, McElveen JE, Sahin U, Luxemburger U, Türeci O, Wiewrodt R, Barnes AC, Robertson JF. *Autoantibodies in lung cancer: possibilities for early detection and subsequent cure*. Thorax. 2008 Mar;63(3):228-33.
- Chapman CJ, Graham F, Healey, Andrea Murray, Peter Boyle, Chris Robertson, Laura J. Peek, Jared Allen, Alison J. Thorpe, Geoffrey Hamilton-Fairley, Celine B. Parsy-Kowalska, Isabel K. MacDonald, William Jewell, Paul Maddison, John F. R. Robertson. *EarlyCDT®-Lung test: improved clinical utility through additional autoantibody assays*. Tumour Biol. 2012 October; 33(5): 1319–1326.
- Dai N, Cao XJ, Li MX, Qing Y, Liao L, Lu XF, Zhang SH, Li Z, Yang YX, Wang D. *Serum APE1 autoantibodies: a novel potential tumor marker and predictor of chemotherapeutic efficacy in non-small cell lung cancer*. PLoS One. 2013;8(3):e58001.
- Desmetz C, Mange A, Maudelonde T, Solassol J. *Autoantibody signatures: progress and perspectives for early cancer detection*. J Cell Mol Med. 2011 Oct;15(10):2013-24.
- Farlow EC, Patel K, Basu S, Lee BS, Kim AW, Coon JS, Faber LP, Bonomi P, Liptay MJ, Borgia JA. *Development of a multiplexed tumor-associated autoantibody-based blood test for the detection of non-small cell lung cancer*. Clin Cancer Res. 2010 Jul 1;16(13):3452-62.
- Rom WN, Goldberg JD, Addrizzo-Harris D, Watson HN, Khilkin M, Greenberg AK, Naidich DP, Crawford B, Eylers E, Liu D, Tan EM. *Identification of an autoantibody panel to separate lung cancer from smokers and nonsmokers*. BMC Cancer. 2010 May 26;10:234.

Shan Q, Lou X, Xiao T, Zhang J, Sun H, Gao Y, Cheng S, Wu L, Xu N, Liu S. *A cancer/testis antigen microarray to screen autoantibody biomarkers of non-small cell lung cancer*. Cancer Lett. 2013 Jan 1;328(1):160-7.

Solassol J, Maudelonde T, Mange A, Pujol JL. Clinical relevance of autoantibody detection in lung cancer. J Thorac Oncol. 2011 May;6(5):955-62.

Ye L, Li X, Sun S, Guan S, Wang M, Guan X, Lee KH, Wei J, Liu B. *A study of circulating anti-CD25 antibodies in non-small cell lung cancer*. Clin Transl Oncol. 2012 Dec 21.

Zhang Y, Ying X, Han S, Wang J, Zhou X, Bai E, Zhang J, Zhu Q. *Autoantibodies against insulin-like growth factor-binding protein-2 as a serological biomarker in the diagnosis of lung cancer*. Int J Oncol. 2013 Jan;42(1):93-100.

Yao Y, Fan Y, Wu J, Wan H, Wang J, Lam S, Lam WL, Girard L, Gazdar AF, Wu Z, Zhou Q. *Potential application of non-small cell lung cancer-associated autoantibodies to early cancer diagnosis*. Biochem Biophys Res Commun. 2012 Jul 6;423(3):613-9.