

APPLICATION GUIDE

TruePLEX™ Antibody Profiling Array: Human Prostate Cancer I

Part Number: AP100010

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

Common Name	Gene Symbol	Gene Name
c-myc	MYC	v-myc myelocytomatosis viral oncogene homolog (avian)
IMP1	IGF2BP1	Insulin-like growth factor 2 mRNA binding protein 1
p53	TP53	Tumor protein p53
p62, IMP2	IGF2BP2	Insulin-like growth factor 2 mRNA binding protein 2
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
mportant Information	4
Recombinant Proteins	5
Assay Protocol	6
Adding Additional Protein Bead Arrays	6
Froubleshooting	6
Additional Breast Cancer Markers available as custom orders Error! Bookma	rk not defined.6
References	6



Ordering Information

TruePlex™ Human Antibody Profiling Kit (96 tests) Catalog #: AP100001

TruePlex[™] Antibody Profiling Arrays for prostate cancer:

Human Prostate Cancer I 5-plex (96 tests) Catalog # AP1000010	c-myc (MYC), IMP1 (IGF2BP1), p53 (TP53), IMP2 (p62, IGF2BP2) and surviving (BIRC5)	
Human Prostate Cancer II 5-plex (96 tests) Catalog # AP1000011	p90 (TFRC), PSA(KLK3), RACE (AMACR), SSX2, SSX4	
Human Prostate Cancer III 5-plex (96 tests) Catalog # AP1000012	HER2/neu (ERBB2), clusterin (CLU), Hsp 70 (HSPA1A), Hsp 71 (HSPA8), NY-ESO-1 (CTAG1B)	
Human Prostate Cancer IV 5-plex (96 tests) Catalog # AP1000013	MCP1 (CCL2), TTLL12, caldesmon 1 (CALD1), PARK7, TARDBP	

Additional panels are available for breast, pancreatic, lung, 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 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 - TRTRPLEQKLISEEDLAANDILDYKDDDDKV

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	Full Name	Common Name	OriGene Protein SKU
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	c-myc	TP301611
IGF2BP1	Insulin-like growth factor 2 mRNA binding protein 1	IMP1	TP316226
TP53	Tumor protein p53	p53	TP300003
IGF2BP2	Insulin-like growth factor 2 mRNA binding protein 2	p62, IMP2	TP305673
BIRC5	Baculoviral IAP repeat containing 5	Survivin	TP305935

Additional prostate cancer markers are available. Contact OriGene for a complete list. (<u>assays@origene.com</u>).



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

Autoantibody signatures: a promising new test for prostate cancer. Harvard Mens Health Watch. 2007 May;11(10):7.

Bradford TJ, Wang X, Chinnaiyan AM. Cancer immunomics: using autoantibody signatures in the early detection of prostate cancer. Urol Oncol. 2006 May-Jun;24(3):237-42.

Ehrlich JR, Caiazzo RJ Jr, Qiu W, Tassinari OW, O'Leary MP, Richie JP, Liu BC. A native antigen "reverse capture" microarray platform for autoantibody profiling of prostate cancer sera. Proteomics Clin Appl. 2007 May;1(5):476-85.

Izhak L, Wildbaum G, Weinberg U, Shaked Y, Alami J, Dumont D, Friedman B, Stein A, Karin N. *Predominant expression of CCL2 at the tumor site of prostate cancer patients directs a selective loss of immunological tolerance to CCL2 that could be amplified in a beneficial manner.* J Immunol. 2010 Jan 15;184(2):1092-101.

Larsson A, Ronquist G, Wülfing C, Eltze E, Bettendorf O, Carlsson L, Nilsson BO, Semjonow A. *Antiprostasome antibodies: possible serum markers for prostate cancer metastasizing liability*. Urol Oncol. 2006 May-Jun;24(3):195-200.

Massoner P, Lueking A, Goehler H, Höpfner A, Kowald A, Kugler KG, Amersdorfer P, Horninger W, Bartsch G, Schulz-Knappe P, Klocker H. *Serumautoantibodies for discovery of prostate cancer specific biomarkers*. Prostate. 2012 Mar;72(4):427-36.

McNeel DG, Nguyen LD, Storer BE, Vessella R, Lange PH, Disis ML. Antibody immunity to prostate cancer associated antigens can be detected in the serum of patients with prostate cancer. J Urol. 2000 Nov;164(5):1825-9.



O'Rourke DJ, DiJohnson DA, Caiazzo RJ Jr, Nelson JC, Ure D, O'Leary MP, Richie JP, Liu BC. *Autoantibody signatures as biomarkers to distinguish prostate cancer from benign prostatic hyperplasia in patients with increased serum prostate specific antigen*. Clin Chim Acta. 2012 Mar 22;413(5-6):561-7.

Ronquist KG, Carlsson L, Ronquist G, Nilsson S, Larsson A. *Prostasome-derived proteins capable of eliciting an immune response in prostate cancer patients*. Int J Cancer. 2006 Aug 15;119(4):847-53.

Xie C, Kim HJ, Haw JG, Kalbasi A, Gardner BK, Li G, Rao J, Chia D, Liong M, Punzalan RR, Marks LS, Pantuck AJ, de la Taille A, Wang G, Mukouyama H, Zeng G. *A novel multiplex assay combining autoantibodies plus PSA has potential implications for classification of prostate cancer from non-malignant cases*. J Transl Med. 2011 Apr 19;9:43.

Zhang JY, Casiano CA, Peng XX, Koziol JA, Chan EK, Tan EM. Enhancement of antibody detection in cancer using panel of recombinant tumorassociated antigens. Cancer Epidemiol Biomarkers Prev. 2003 Feb;12(2):136-43.