

APPLICATION GUIDE

TruePLEX™ Antibody Profiling Array: Human Breast Cancer III

Part Number: AP100008

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

Common Name	Gene Symbol	Gene Name
FKBP52	FKBP4	FK506 binding protein 4
HSPA14	HSPA14	heat shock 70kDa protein 14
HSP-60 / chaperonin	HSPD1	heat shock 60kDa protein 1
cyclophilin A	PPIA	peptidylprolyl isomerase A
Thioredoxin peroxidase 1	PRDX2	peroxiredoxin 2

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

For Research Use Only

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Ordering Information

TruePlex™ Human Antibody Profiling Kit (96 tests)

Catalog #: AP100001

TruePlex™ Antibody Profiling Arrays for breast cancer:

Human Breast Cancer I 5-plex (96 tests) Catalog # AP100006	P53 (TP53) , c-myc (MYC), Her2/ERBB2 , NY-ESO-1 (CTAG1), BRCA2
Human Breast Cancer II 5-plex (96 tests) Catalog # AP100007	MUC1, BIRC5 (Survivin), IFG2BP2((IMP2), IGF2BP1 (IMP1), CDKN2A (p16)
Human Breast Cancer III 5-plex (96 tests) Catalog # AP100008	FKBP4 (FKBP52), HSPA14, HSP-60 (chaperonin), cyclophilin A (PPIA), Thioredoxin peroxidase 1 (PRDX2)
Human Breast Cancer IV 5-plex (96 tests) Catalog # AP100009	ALG-2-interacting protein 1 (PDCD6IP), BCAT2 (DBT), CKI-epsilon (CSNK1E), p21-Rac3 (RAC3), SF3a120 (SF3A1)

Additional panels are available for prostate, 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 - TRTRPEQKLISEEDLAANDILDYKDDDDKV

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.

Bead #	Gene Symbol	Full Name	Common Name	OriGene Protein SKU
12	FKBP4	FK506 binding protein 4	FKBP52	TP300713
20	HSPA14	heat shock 70kDa protein 14	HSPA14	TP318858
28	HSPD1	heat shock 60kDa protein 1	HSP-60 / chaperonin	TP760006
52	PPIA	peptidylprolyl isomerase A	cyclophilin A	TP303307
62	PRDX2	peroxiredoxin 2	Thioredoxin peroxidase 1	TP309548

Additional breast cancer markers are available. Contact OriGene for a complete 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

Alper O, Stetler-Stevenson WG, Harris LN, Leitner WW, Ozdemirli M, Hartmann D, Raffeld M, Abu-Asab M, Byers S, Zhuang Z, Oldfield EH, Tong Y, Bergmann-Leitner E, Criss WE, Nagasaki K, Mok SC, Cramer DW, Karaveli FS, Goldbach-Mansky R, Leo P, Stromberg K, Weil RJ. *Novel anti-filamin-A antibody detects a secreted variant of filamin-A in plasma from patients with breast carcinoma and high-grade astrocytoma.* Cancer Sci. 2009 Sep;100(9):1748-56.

Anderson KS, Sibani S, Wallstrom G, Qiu J, Mendoza EA, Raphael J, Hainsworth E, Montor WR, Wong J, Park JG, Lokko N, Logvinenko T, Ramachandran N, Godwin AK, Marks J, Engstrom P, Labaer J. *Protein microarray signature of autoantibody biomarkers for the early detection of breast cancer.* J Proteome Res. 2011 Jan 7;10(1):85-96.

Carter D, Dillon DC, Reynolds LD, Retter MW, Fanger G, Molesh DA, Sleath PR, McNeill PD, Vedvick TS, Reed SG, Persing DH, Houghton RL. *Serum antibodies to lipophilin B detected in late stage breast cancer patients.* Clin Cancer Res. 2003 Feb;9(2):749-54.

Chapman C, Murray A, Chakrabarti J, Thorpe A, Woolston C, Sahin U, Barnes A, Robertson J. *Autoantibodies in breast cancer: their use as an aid to early diagnosis.* Ann Oncol. 2007 May;18(5):868-73.

Conroy SE, Gibson SL, Brunström G, Isenberg D, Luqmani Y, Latchman DS. *Autoantibodies to 90 kD heat-shock protein in sera of breast cancer patients.* Lancet. 1995 Jan 14;345(8942):126.

Desmetz C, Bascoul-Mollevi C, Rochaix P, Lamy PJ, Kramar A, Rouanet P, Maudelonde T, Mangé A, Solassol J. *Identification of a new panel of serum autoantibodies associated with the presence of in situ carcinoma of the breast in younger women.* Clin Cancer Res. 2009 Jul 15;15(14):4733-41.

Desmetz C, Bibeau F, Boissière F, Bellet V, Rouanet P, Maudelonde T, Mangé A, Solassol J. *Proteomics-based identification of HSP60 as a tumor-associated antigen in early stage breast cancer and ductal carcinoma in situ.* J Proteome Res. 2008 Sep;7(9):3830-7.

Looi K, Megliorino R, Shi FD, Peng XX, Chen Y, Zhang JY. *Humoral immune response to p16, a cyclin-dependent kinase inhibitor in human malignancies.* Oncol Rep. 2006 Nov;16(5):1105-10.

Lu H, Goodell V, Disis ML. *Humoral immunity directed against tumor-associated antigens as potential biomarkers for the early diagnosis of cancer.* J Proteome Res. 2008 Apr;7(4):1388-94.

Mangé A, Lacombe J, Bascoul-Mollevi C, Jarlier M, Lamy PJ, Rouanet P, Maudelonde T, Solassol J. Serum autoantibody signature of ductal carcinoma in situ progression to invasive breast cancer. Clin Cancer Res. 2012 Apr 1;18(7):1992-2000.

Pupa SM, Forti S, Balsari A, Ménard S. *Humoral immune response for early diagnosis of breast carcinoma.* Ann Oncol. 2002 Mar;13(3):483.

Salama O, Herrmann S, Tziknovsky A, Piura B, Meirovich M, Trakht I, Reed B, Lobel LI, Marks RS. *Chemiluminescent optical fiber immunosensor for detection of autoantibodies to ovarian and breast cancer-associated antigens.* Biosens Bioelectron. 2007 Feb 15;22(7):1508-16.

Sorokine I, Ben-Mahrez K, Bracone A, Thierry D, Ishii S, Imamoto F, Kohiyama M. *Presence of circulating anti-c-myb oncogene product antibodies in human sera.* Int J Cancer. 1991 Mar 12;47(5):665-9.

Yagihashi A, Ohmura T, Asanuma K, Kobayashi D, Tsuji N, Torigoe T, Sato N, Hirata K, Watanabe N. *Detection of autoantibodies to survivin and livin in sera from patients with breast cancer.* Clin Chim Acta. 2005 Dec;362(1-2):125-30.

Ye H, Sun C, Ren P, Dai L, Peng B, Wang K, Qian W, Zhang J. *Mini-array of multiple tumor-associated antigens (TAAs) in the immunodiagnosis of breast cancer.* Oncol Lett. 2013 Feb;5(2):663-668.

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

Zhong L, Ge K, Zu JC, Zhao LH, Shen WK, Wang JF, Zhang XG, Gao X, Hu W, Yen Y, Kernstine KH. *Autoantibodies as potential biomarkers for breast cancer.* Breast Cancer Res. 2008;10(3):R40.