

# APPLICATION GUIDE

## TruePLEX™ Antibody Profiling Array: Human Ovarian Cancer I

Part Number: AP100025

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

Name	Gene Symbol	Full Name
HOMER2	HOMER2	Homer homolog 2 (Drosophila)
PIK3AP1	PIK3AP1	Phosphoinositide-3-kinase adaptor protein 1
LAGE-1	CTAG2	Cancer/testis antigen 2
p53	TP53	Tumor protein p53
NY-ESO-1	CTAG1B/CTAG1A	Cancer/testis antigen 1
Yo Protein	CDR2	Cerebellar degeneration-related protein 2, 62kDa
Ri Protein	NOVA1	Neuro-oncological ventral antigen 1
Ri Protein	NOVA2	Neuro-oncological ventral antigen 2
S100A7	S100A7	S100 calcium binding protein A7
AASDHPPT	AASDHPPT	aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase
Mesothelin	MSLN	mesothelin

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:

Human Ovarian Cancer I (96 tests) Catalog #: AP100025

Human Ovarian Cancer II (96 tests) Catalog #: AP100026

Human Ovarian Cancer III (96 tests) Catalog #: AP100027

Human Ovarian Cancer IV (96 tests) Catalog #: AP100028

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](mailto: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	Name	OriGene Protein SKU
HOMER2	Homer homolog 2 (Drosophila)	TP304165
PIK3AP1	Phosphoinositide-3-kinase adaptor protein 1	TP314125
CTAG2	Cancer/testis antigen 2	TP311659
TP53	Tumor protein p53	TP300003 TP325604
CTAG1B/CTAG1A	Cancer/testis antigen 1	TP313318 TP316285
CDR2	Cerebellar degeneration-related protein 2, 62kDa	TP760128
NOVA1	Neuro-oncological ventral antigen 1	TP310407
NOVA2	Neuro-oncological ventral antigen 2	TP316200
S100A7	S100 calcium binding protein A7	TP304490
AASDHPPT	aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase	TP305091
MSLN	mesothelin	TP302532

Additional ovarian cancer markers may be available. Contact OriGene for an updated list. ([assays@origene.com](mailto: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](mailto:assays@origene.com) for more information.

## Troubleshooting

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

## References

Drlicke M, Bianchi G, Bogliun G, et al. Antibodies of the anti-Yo and anti-Ri type in the absence of paraneoplastic neurological syndromes: a long-term survey of ovarian cancer patients. *J Neurol* 1997; 244:85-89.

Gagnon A, Kim JH, Schorge JO, et al. Use of a combination of approaches to identify and validate relevant tumor-associated antigens and their corresponding autoantibodies in ovarian cancer patients. *Clin Cancer Res* 2008; 14:764-71.

Gunawardan CG, Memari N, Diamandis EP. Identifying novel autoantibody signatures in ovarian cancer using high-density protein microarrays. *Clinical Biochemistry* 2009; 42:426-29.

Hellstrom I, Friedman E, Verch T, Yang Y, Korach J, Jaffar J, et al. Antimesothelin antibodies and circulating mesothelin relate to the clinical state in ovarian cancer patients. *Cancer Epidemiol Biomarkers Prev* 2008;17:1520–6

Hudson ME, Pozdnyakova I, Haines K, et al. Identification of differentially expressed proteins in ovarian cancer using high-density protein microarrays. *PNAS* 2009; 104:17494-99.

Korneeva I, Bongiovanni AM, Girotra M, et al. Serum antibodies to the 27-kd heat shock protein in women with gynecologic cancers. *Am J Obstet Gynecol* 2000; 183:18-21.

Luo LY, Herrera I, Soosaipillai A, Diamandis EP. Identification heat shock protein 90 and other proteins as tumour antigens by serological screening of an ovarian carcinoma expression library. *British J Cancer* 2002; 87:339-43.

Muro Y, Ogawa Y, Kato Y, et al. Autoantibody to thioredoxin reductase in an ovarian cancer patient. *Biochem Biophys Res Comm* 1998; 242:267-71.

Reuschenbch M, Doeberitz M, Wentzensen M. A systematic review of humoral immune responses against tumor antigens. *Cancer Immunol Immunother* 2009; 58:1535-44.

Sacha Gnjjatic, Erika Ritter, Markus W. Büchler, Nathalia A. Giese, Benedikt Brors, Claudia Frei, Anne Murray, Niels Halama, Inka Zörnig, Yao-Tseng Chen, Christopher Andrews, Gerd Ritter, Lloyd J. Old, Kunle Odunsi, Dirk Jäger. *Proc Natl Acad Sci U S A*. 2010 March 16; 107(11): 5088–5093.

Stokert E, Jager E, Chen YT, et al. A survey of the humoral immune response of cancer patients to an panel of human tumor antigens. *J Exp Med* 1998; 187:1349-54.

Taylor DD, Gercel-Taylor C, Parker LP. Patient-derived tumor-reactive antibodies as diagnostic markers for ovarian cancer. *Gynecol Oncol*. 2009 October ; 115(1): 112–120.

Tsai-Turton M, Santillan A, Lu D, et al. P53 autoantibodies, cytokine levels and ovarian carciogenesis. *Gynecol Oncol* 2009; 114:12-17.

Yavelsky V, Rohkin S, Shaco-Levy R, et al. Native human autoantibodies targeting GIPCI identify differential expression in malignant tumors of the breast and ovary. *BMC Cancer* 2008; 8:247-58.