APPLICATION GUIDE

TruePLEX™ Antibody Profiling Array: Human Breast Cancer II

Part Number: AP10000X

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

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC1</td>
<td>MUC1</td>
<td>mucin 1, cell surface associated</td>
</tr>
<tr>
<td>Survivin</td>
<td>BIRC5</td>
<td>baculoviral IAP repeat containing 5</td>
</tr>
<tr>
<td>p62, IMP2</td>
<td>IGF2BP2</td>
<td>insulin-like growth factor 2 mRNA binding protein 2</td>
</tr>
<tr>
<td>IMP1</td>
<td>IGF2BP1</td>
<td>insulin-like growth factor 2 mRNA binding protein 1</td>
</tr>
<tr>
<td>p16</td>
<td>CDKN2A</td>
<td>cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)</td>
</tr>
</tbody>
</table>

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 .................................................................................................................... 6
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 breast cancer:

<table>
<thead>
<tr>
<th>Human Breast Cancer I</th>
<th>P53 (TP53 ), c-myc (MYC), Her2/ERBB2, NY-ESO-1 (CTAG1), BRCA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-plex (96 tests)</td>
<td>Catalog # AP100006</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Human Breast Cancer II</th>
<th>MUC1, BIRC5 (Survivin), IFG2BP2 (IMP2), IGF2BP1 (IMP1), CDKN2A (p16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-plex (96 tests)</td>
<td>Catalog # AP100007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Human Breast Cancer III</th>
<th>FKBP4 (FKBP52), HSPA4, HSP-60 (chaperonin), cyclophilin A (PPIA), Thioredoxin peroxidase 1 (PRDX2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-plex (96 tests)</td>
<td>Catalog # AP100008</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Human Breast Cancer IV</th>
<th>ALG-2-interacting protein 1 (PDCD6IP), BCAT2 (DBT), CKI-epsilon (CSNK1E), p21-Rac3 (RAC3), SF3a120 (SF3A1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-plex (96 tests)</td>
<td>Catalog # AP100009</td>
</tr>
</tbody>
</table>

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 -20ºC.

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.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Full Name</th>
<th>Common Name</th>
<th>OriGene Protein SKU</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC1</td>
<td>mucin 1, cell surface associated</td>
<td>MUC1</td>
<td>TP321390</td>
</tr>
<tr>
<td>BIRC5</td>
<td>baculoviral IAP repeat containing 5</td>
<td>Survivin</td>
<td>TP305935</td>
</tr>
<tr>
<td>IGF2BP2</td>
<td>insulin-like growth factor 2 mRNA binding protein 2</td>
<td>p62, IMP2</td>
<td>TP305673</td>
</tr>
<tr>
<td>IGF2BP1</td>
<td>insulin-like growth factor 2 mRNA binding protein 1</td>
<td>IMP1</td>
<td>TP316226</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)</td>
<td>p16</td>
<td>TP320937</td>
</tr>
</tbody>
</table>

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


