CDI Laboratories

# User Guide

# HuProt™ Human Proteome Microarray v3.1

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## Summary

The HuProt™ human proteome microarray provides the largest number of unique human proteins known to be included on a single slide, allowing thousands of interactions to be profiled in high-throughput.

| | |
|---|---|
| Microarray type | Functional protein microarray |
| Species | Human |
| Production technology | Non-contact piezoelectric inkjet |
| Slide type | Coated glass* |
| Detection method | Fluorescence |
| Content | Recombinant human & mouse proteins** |

*The HuProt™ human proteome microarray is available on two types of glass surfaces: PATH® and SuperEpoxy2™. Please contact us to discuss your needs.

** Please refer to the documentation accompanying the microarrays for specific information.

The HuProt™ version 3.1 microarray contains >19,500 unique proteins. This content encompasses 16,152 unique human genes (~81% of the proteome) and 124 unique mouse gene symbols. Recombinant proteins are expressed in the yeast *S. cerevisiae*, purified, and printed on glass slides in duplicate, along with control proteins (page 2)

These expressed recombinant proteins are N-terminal GST and RGS-His6-tagged, and the quality of each microarray batch is determined by GST immunoblotting (98% of all proteins show GST signals significantly higher than negative controls). For most applications, we print the microarray on glass slides that are coated with an ultra-thin layer of nitrocellulose film for the non-covalent, yet irreversible, capture of active proteins to the surface.

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**Summary,** continued from page 1.

HuProt™ V3.1 Control Spots

| Control | Function |
|---|---|
| H1 - Histone H1<br>H2 (A+B) - Histone H2A and H2B mixture<br>H3 - Histone H3<br>H4 - Histone H4 | The histones are non-specific binding proteins used as positive controls for various assays, including serum profiling, antibody specificity assay, protein, DNA, RNA-binding assays, etc. |
| IgG488/594 | Alexa Fluor 488/594 labeled IgG, positive control and landmarks for fluorescent detection in 488/594 channels. |
| Rhodamine + IgG 647 | Rhodamine + Alexa Fluor 647 labeled IgG, positive control and landmarks for fluorescent detection in 532/635 channels. |
| Anti-human IgA | A positive control for human serum/plasma IgA profiling. |
| Mouse-anti-biotin | Detects biotin labeled protein probes and serves as a control for anti-mouse antibody detection reagent. |
| Rabbit-anti-biotin | Detects biotin labeled protein probes and serves as a control for anti-rabbit antibody detection reagent. |
| BSA - Bovine serum albumin | A negative control for non-specific protein interactions |
| Biotin-BSA - biotinylated BSA | A positive control for interaction with streptavidin-labeled detection reagent |
| Mouse IgM | Positive control for anti-mouse IgM detection. |
| RanBP2deltaFG | E3 SUMO-Protein Ligase, a positive control for the SUMOylation assay |
| hMDM2 | E3 Ubiquitin Protein Ligase, a positive control for the ubiquitinylation assay |
| ERα | Estrogen receptor alpha. A positive control for ligand-binding assays. |
| Human IgM | A positive control for human serum/plasma IgM profiling. |
| Human IgG 1.5625 ng/µl<br>Human IgG 6.25 ng/µl<br>Human IgG 25 ng/µl<br>Human IgG 100 ng/µl | The human IgG gradient serves as a positive control for human serum/plasma IgG profiling, and is used for Robust-Linear-Model Normalization in data analysis. |
| Anti-Human IgG 1.5625 ng/µl<br>Anti-Human IgG 6.25 ng/µl<br>Anti-Human IgG 25 ng/µl<br>Anti-Human IgG 100 ng/µl | The Anti-human IgG gradient serves as a positive control for human serum/plasma IgG profiling, and is used for Robust-Linear-Model Normalization in data analysis. |
| GST (glutathione S-transferase) 10 ng/µl<br>GST (glutathione S-transferase) 50 ng/µl<br>GST (glutathione S-transferase) 100 ng/µl<br>GST (glutathione S-transferase) 200 ng/µl | The glutathione S-transferase (GST) protein gradient serves as a negative control, and is used for background and statistical significance calculations. |
| Buffer | Printing buffer only, negative control |

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## Storage and Handling

**IMPORTANT:** New HuProt™ microarrays must be stored in an ultra cold and dry environment.

HuProt™ microarrays are shipped in closed plastic slide holders on dry ice, or with gel coolant sheets. Upon arrival, microarrays should immediately be stored at -80°C.

To ensure the best performance from the HuProt™ microarray:

- Wear gloves at all times
- Do not touch the active surface of the microarray (the surface where the bar code label is attached) with hands, with pipette tips or with tweezers. The active surface should face up at all times.
- Handle microarrays only along the edge near the barcode, using tweezers.
- Do not let the HuProt™ microarray dry out at any time during the assay.
- Glass cover slips are used to minimize evaporation of the sample when conducting low volume assays. Be very careful when adding a cover slip to the active surface of the microarray, to prevent scratching or smudging the microarray surface. Likewise, when the assay is completed, very carefully remove the cover slip prior to washing the arrays. One alternative is to immerse the covered microarray in a large volume of wash buffer, and then let the cover slip float off.

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## Overview - Key Steps for HuProt™ Microarray Use

Prepare a cold environment

- It is critical to keep the microarrays ultra-cold and ultra-dry right until they are used. HuProt™ microarrays should be stored at -80°C. Before removing HuProt™ microarrays out of storage, place a layer of dry ice pellets inside a styrofoam box with a lid and cover tightly. Remove the plastic slide holders containing HuProt™ microarrays from the freezer and place it lengthwise on top of the dry ice. Add an additional layer of dry ice pellets or a sheet of frozen gel coolant on top of the plastic slide holders. Do not let condensate form on the surface before using the microarrays.

Prepare 4-well plates for the blocking step

- 4-well plates are used to hold the HuProt™ microarrays during blocking, during the assay (high volume samples) and during the washing steps (e.g. *Nunc* 4-well rectangular dishes, Fisher Scientific No.12-565-495). Each compartment of the 4-well plates holds one microarray. To minimize cross-contamination of samples during the assay, some use no more than 2 microarrays in each 4-well plate, and keep an empty compartment in between the microarrays.
- Add 3.0 ml of blocking buffer to each compartment of the 4-well plates (e.g. * Nunc* 4-well rectangular dishes, Fisher Scientific No.12-565-495).

**IMPORTANT:** Handle the microarray only on the edges of the glass, at the end where the bar code is attached. Do not to touch the active surface of the microarray.

Block the HuProt™ microarrays

- The active surface of the HuProt™ microarray is the surface with the barcode. Carefully use fine-nosed tweezers to remove one microarray from the plastic slide holder that is resting on dry ice. Immediately submerge the HuProt™ microarray, with the active surface facing up, in a 4-well plate containing 3.0 ml blocking buffer. Incubate with gentle shaking for 5 min.
- Remove the blocking buffer from a corner of the 4-well plate via aspiration. Use the microarrays immediately in the assay of choice (see below).

**IMPORTANT:** Blocking solution recipes vary by protocol—please refer to the relevant protocol for the correct recipe.

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## Sample Preparation and Assay

- High volume assays: If a high volume of test sample is available, dilute it in 3.0 ml of buffer. Add this to one compartment of a 4-well plate and immerse the HuProt™ microarray face up in the solution (no coverslip is needed).
- For low volume assays (100 - 300 µl), evaporation must be minimized. Add the entire diluted test sample to the active surface of the microarray, and carefully cover the microarray with a new cover slip. Place the covered microarray in a humidification chamber.

**NOTE:** An empty yellow tip box may be used to create a humidification chamber. Place wet clean room wipes or paper towels in the base of a yellow tip box. Replace the empty plastic tip rack above the base, over the wet clean room wipes or towels. Carefully place up to four cover slipped HuProt™ microarrays on the rack, and close the lid.

## Washing

- When assays are completed, wash the microarrays in 4-well plates. For low volume reactions, the cover slip must be first be carefully removed, without touching or scratching the active surface of the microarray. Immerse the covered microarray in a compartment of a 4-well plate containing 4.0 ml of wash buffer (the buffer will vary depending on the assay). Using fine-nosed tweezers, carefully lift off the cover slip, starting from the barcoded end of the microarray. An alternative is to float off the cover slip by immersing the microarray in a larger volume of wash buffer.
- For high volume samples, carry out the washes in the same 4-well plates in which the assays and blocking were conducted.

**IMPORTANT:** Do not let the HuProt™ microarrays sit for long periods in wash buffer. Proceed to the drying stage immediately.

**IMPORTANT:** If fluorescent probes are used at any point, minimize exposure to light to prevent quenching. Cover all 4-well plates or other containers holding the microarrays or labeled probes with aluminum foil, or use a lightproof storage container.

## Scan and Store the Microarrays

- After the washes are completed, scan the microarrays immediately (highly preferred) or store them in a lightproof microscope slide box at -20°C. During storage, the active surface of the microarray should not touch any other surfaces, including other microarrays.

**IMPORTANT:** Microarrays must be scanned within 3 days after the assay is performed.

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## Additional Reagents and Materials

### Materials

Secondary Antibodies for Detection Step:

- The quality of commercially available secondary antibodies can vary widely. Please test all secondary antibodies thoroughly before conducting your assay (the secondary antibodies listed in this manual are provided as examples).
- Create grid alignment for data analysis using the GST-tagged proteins printed on the HuProt™ microarray as a visual reference.

**IMPORTANT**: We do not recommend doing the primary assay and anti-GST staining on the microarray at the same time. First conduct the primary assay, and scan the microarray data. After this, anti-GST probing can be done using an anti-Glutathione-S-Transferase antibody (e.g. Glutathione-S-Transferase, *S. japonicum* form; MED Millipore, cat no. AB3282). For this step, use a secondary antibody with a detection wavelength that is different from the wavelength used to detect the primary reaction.

**NOTE:** The GST signals obtained after your primary assay should not be used as a measure of the HuProt™ microarray quality, as all proteins on the microarray will become inactive after the primary assay. If too much anti-Glutathione-S-Transferase antibody is added, this may result in a very high background and may negatively affect data analysis.

**Additional materials and equipment for incubations/assays:**

Aluminum foil

Automatic pipettes

Cleanroom wipes (preferred) or paper towels

Micropipettes

Orbital shaker

Sterile disposable micropipette tips

Sterile serological pipettes

Fine nosed tweezers

Vacuum system

Vortex

Plastic 4-well plates to store HuProt™ microarrays during the blocking, reaction and washing steps (e.g. Thermo Scientific *Nunc* Dishes, Rectangular 4-Well, No.12-565-495)

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BD Shoulder Cover Microscope Slide Box (lightproof; e.g. Fisher Scientific cat. no. 22-167-403)

Cover slips (e.g. LifterSlip by Thermo Scientific, cat. no. 25X60I24789001LS, and Grace Bio-Labs HS6024 hybrislip 60X24MM, Fisher Scientific cat. no. NC9296662)

Humidification chamber (for low incubation volumes between 100-300 µl) or a modified empty yellow tip box

**Additional Materials and Equipment for Data Analysis:**

Microarray scanner (e.g. Molecular Devices GenePix 4000B) and computer

Microarray analysis program (e.g. GenePix Pro 6.1)

**General Reagents:**

Bovine Serum Albumin (IgG-Free, Protease-Free; Jackson ImmunoResearch Laboratories)

KCl

NaCl

Tris base

Tween-20

DTT (dithiothreitol) NOTE: DTT is not stable in solution. Only freshly-made DTT solutions should be used in buffers.

Triton-X 100

HEPES

Urea

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## Assays Using the HuProt™ Human Proteome Microarray

### I. Monoclonal Antibody Specificity Determination Assay

**I.1** Storage:

- Store HuProt™ microarrays inside closed plastic slide holders at -80°C or on a layer of dry ice, right until the blocking step. The active surface of the HuProt™ microarray is the surface with the barcode.

**IMPORTANT:** It is critical to keep the HuProt™ microarrays ultra-cold and ultra-dry before use. Do not let condensate form on the surface before the microarray is used.

**I.2** Blocking:

- Add 3.0 ml of blocking solution (5% BSA in 1X TBS-T) to each compartment of the 4-well plates. Carefully use fine-nosed tweezers to remove one microarray from the plastic slide holder that is resting on dry ice. Immediately submerge the HuProt™ microarray, active surface up, in a compartment of the 4-well plate containing blocking buffer. Incubate at room temperature, with gentle shaking for 1.5-2 hrs.

**I.3** Sample Preparation: Prepare the primary monoclonal antibody for testing

- **A.** Low volume reaction: If only a limited amount of the primary antibody being tested is available, dilute the primary antibody to a concentration of 1 µg/ml in 300 µl blocking buffer. Store on ice.
- **B.** High volume reaction: If the amount of primary antibody to be tested is not limited, dilute the primary antibody to a concentration of 1 µg/ml in 3.0 ml blocking buffer. Store on ice.
- **C.** Supernatant: If the antibody source is a supernatant, dilute the supernatant 1:12 in blocking buffer to a final volume of 300 µl. Store on ice.

**NOTE:** This dilution assumes that the antibody concentration in the supernatant is 5 µg /ml. Please base your dilution on the actual antibody concentration of your sample.

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**I.4** Assay: Add monoclonal antibody sample to HuProt™ microarrays

- Carefully use aspiration to remove the blocking buffer from a corner of the 4-well plates that contain the immersed human proteome microarrays. Do not touch the microarray surface.
- **A & C.** For low volume samples (100 µl to 300 µl), carefully pipette the prepared primary monoclonal antibody onto the active surface of the blocked HuProt™ microarray (barcode side up). Do not let the pipette tip touch the microarray surface. Cover the microarray with a cover slip to minimize evaporation and place in a humidification chamber. Incubate with gentle shaking on an orbital shaker at room temperature for 1 hr. **IMPORTANT:** Use at least 100 µl of sample.
- **B.** For high volume samples (up to 3.0 ml), carefully add the sample to the active (top) surface of the microarray in the 4-well plate. Be careful not to touch the surface of the microarray. Incubate with gentle shaking on an orbital shaker at room temperature for 1 hr.

**I.5** Washing:

- Upon completing the assay, the microarrays are washed in 4-well plates. For low volume reactions carried out in a humidification chamber, the cover slip must be removed prior to washing. Immerse the covered microarray in a compartment of a 4-well plate containing 4.0 ml of wash buffer. Using fine-nosed tweezers, carefully lift off the cover slip, starting from the barcoded end of the microarray, taking care to not touch or scratch the active surface of the microarray. An alternative method is to float the cover slip off by immersing the microarray in a larger volume of wash buffer.
- Rinse each slide briefly with 4.0 ml of 1X TBS-T. Remove the buffer after rinsing using aspiration.
- Add 4.0 ml of 1X TBS-T and incubate with gentle shaking at room temperature for 10 min, then remove the buffer by aspiration. Repeat for a total of three washes.

**I.6** Detection: Add Secondary Antibodies

- Dilute secondary/detection antibody in blocking buffer to the manufacturer-recommended concentration.
- Add 3.0 ml of diluted secondary antibody to each compartment in a 4-well plate containing the HuProt™ microarrays.
- Cover the 4-well plates with aluminum foil and incubate at room temperature for 1 hr with gentle shaking.

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Reference:

Jeong JS *et al.* (2012) Rapid identification of monospecific monoclonal antibodies using a human proteome microarray. *Mol Cell Proteomics* **11** 10.1074/mcp.O111.016253.

Liu S *et al.* (2015) Characterization of monoclonal antibody's binding kinetics using oblique-incidence reflectivity difference approach. *MAbs* **7**(1):110-9. doi: 10.4161/19420862.2014.985919.



- Remove the buffer containing the secondary antibody from a corner of the 4-well plate by aspiration.

**IMPORTANT:** After adding the secondary antibody, store the microarrays in the dark. For all incubation and washing steps below, cover the 4-well plates containing HuProt™ microarrays with aluminum foil to minimize light exposure, which can quench the fluorescence.

**I.7** Washing:

- Rinse each slide briefly with 4.0 ml of 1X TBS-T. Remove the buffer after washing using aspiration.
- Add 4.0 ml of 1X TBS-T to each compartment of the 4-well plate, cover with foil, and incubate with gentle shaking for 10 min at room temperature.
- Briefly rinse the microarrays three times with 0.1X TBS.

**I.8** Drying:

- Place clean room wipes or paper towels on the bottom of a black microscope slide box (will hold several microarrays) or prepare plastic conical 50-ml tubes (each will hold one microarray).
- Remove the HuProt™ microarray from the 4-well plates and tap the edge lightly on a clean room wipe to remove excess fluid. Do not touch the active surface of the microarray. Carefully slot the microarrays into the microscope slide box – the microarrays will be perpendicular to the paper towels lining the base of the box. If you are using 50-ml tubes, carefully slide a single microarray lengthwise into the tube.
- To remove excess fluid, spin the microscope slide box or the 50-ml tubes containing the microarrays in a centrifuge at 800 rpm for 3 min (spinning at higher speeds may break the microarray). After centrifugation, carefully remove the HuProt™ microarrays and discard the clean room wipes.

**I.9** Scanning:

- The HuProt™ microarrays can be scanned immediately (highly preferred) or stored at -20°C in a lightproof box.

**IMPORTANT:** Microarrays must be scanned within 3 days after the assay is performed.

Appendix for Monoclonal Antibody Specificity Determination:

**Recipes**

- 1X TBS-T Buffer: TBS pH 7.5, 0.1% Tween-20 (see General Appendix, Buffers section)
- Blocking Solution: 5% BSA [w/v] in 1X TBS-T Buffer: Dissolve 5 g of Bovine Serum Albumin (IgG-Free, Protease-Free) in 60 ml of 1X TBS-T. Complete to 100 ml with 1X TBS-T and filter before use.

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## II. Monoclonal Antibody Specificity Determination Assay Using Denatured Arrays

**II.1** Storage:

- Store HuProt™ microarrays inside closed plastic slide holders at -80°C or on a layer of dry ice, right until the blocking step. The active surface of the HuProt™ microarray is the surface with the barcode.

**IMPORTANT:** It is critical to keep the HuProt™ microarrays ultra-dry before use. Do not let condensate form on the surface before the microarray is used.

**II.2** Blocking:

- Add 3.0 ml of blocking solution (5% BSA in 1X TBS-T) to each compartment of the 4-well plates. Carefully use fine-nosed tweezers to remove one microarray from the plastic slide holder that is resting on dry ice. Immediately submerge the HuProt™ microarray, active surface up, in a compartment of the 4-well plate containing blocking buffer. Incubate at room temperature, with gentle shaking for 1.5-2 hrs.

**II.3** Denature the HuProt™ Array:

- Add 3.0 ml of denaturation buffer (9M Urea with 5 mM DTT) to each compartment of the 4-well plates. Incubate at room temperature for 20 min WITHOUT shaking.
- Wash in 1X TBS-T for 5 min with gentle shaking. Carefully pour off the washing buffer or remove it by aspiration. Add 3.0 ml fresh 1X TBS-T and repeat, for a total of 3 washes.

**II.4** Sample Preparation: Prepare the primary monoclonal antibody for testing

- **A.** Low volume reaction: If the primary antibody being tested is only available in a limited amount, dilute the primary antibody to a concentration of 1 µg/ml in 300 µl blocking buffer. Store on ice.
- **B.** High volume reaction: Dilute the primary antibody to a concentration of 1 ug/ml in 3.0 ml blocking buffer. Store on ice.
- **C.** Supernatant: If the antibody source is in supernatant form, dilute the supernatant 1:12 in blocking buffer, to a final volume of 300 µl. Store on ice.

**NOTE:** This dilution assumes that the concentration of antibody in the supernatant is 5 µg/ml. Please base your dilution on the actual concentration of antibody in your sample.

**II.5** Assay: Add monoclonal antibody sample to HuProt™ microarrays

- Carefully use aspiration to remove the blocking buffer from a corner of the 4-well plates that contain the immersed human proteome microarrays. Do not touch the microarray surface.

- **A & C:** For low volume samples (100 µl to 300 µl), carefully pipette the prepared primary monoclonal antibody onto the active surface of the blocked HuProt™ microarray (barcode side up). Do not let the pipette tip touch the microarray surface. Cover the microarray with a cover slip to minimize evaporation and place in a humidification chamber. Incubate with gentle shaking on an orbital shaker at room temperature for 1 hr, or at 4°C overnight. **IMPORTANT:** Use a sample of at least 100 µl.
- **B:** For high volume samples (up to 3.0 ml), carefully add the sample to the active (top) surface of the microarray in the 4-well plate. Be careful not to touch the surface of the microarray. Incubate with gentle shaking on an orbital shaker at room temperature for 1 hr, or at 4°C overnight.

**II.6** Washing:

- Upon completing the assay, wash the microarrays in 4-well plates. To wash arrays used to test low volume samples, first immerse the covered microarray in a compartment of a 4-well plate containing 4.0 ml of wash buffer. Using fine-nosed tweezers, carefully lift off the cover slip, starting from the barcoded end of the microarray, taking care to not touch or scratch the active surface of the microarray. An alternative method is to float the cover slip off by immersing the microarray in a larger volume of wash buffer.
- Rinse each slide briefly with 4.0 ml of 1X TBS-T. After rinsing, remove the buffer using aspiration.
- Add 4.0 ml of 1X TBS-T and incubate with gentle shaking at room temperature for 10 min, then remove the buffer by aspiration. Repeat for a total of three washes.

**II.7** Detection: Add Secondary Antibodies

- Dilute secondary/detection antibody in blocking buffer to the manufacturer-recommended concentration.
- Add 3.0 ml of diluted secondary antibody to each compartment of a 4-well plate that contains HuProt™ microarrays.
- Cover the 4-well plates with aluminum foil and incubate at room temperature for 1 hr with gentle shaking.
- Remove the buffer containing the secondary antibody from a corner of the 4-well plate by aspiration.

**IMPORTANT:** After the secondary antibody is added, store the microarrays in the dark. For all incubation and washing steps below, cover the 4-well plates containing HuProt™ microarrays with aluminum foil to minimize light exposure, which can quench the fluorescence.

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**II.8** Washing:

- Rinse each slide briefly with 4.0 ml of 1X TBS-T. After rinsing, remove the buffer using aspiration.
- Add 4.0 ml 1X TBS-T to each compartment of the 4-well plate, cover with foil, and incubate with gentle shaking for 10 min at room temperature. Remove the buffer from a corner of the 4-well plate by aspiration. Repeat for a total of three washes.
- Briefly rinse the microarrays three times with 0.1X TBS.

**II.9** Drying:

- Place clean room wipes or paper towels on the bottom of a black microscope slide box (will hold several microarrays) or prepare plastic conical 50-ml tubes (each will hold one microarray).
- Remove the HuProt™ microarray from the 4-well plates and tap the edge lightly on a clean room wipe to remove excess fluid. Do not touch the active surface of the microarray. Carefully slot the microarrays into the microscope slide box – the microarrays will be perpendicular to the paper towels lining the base of the box. If you are using 50 ml tubes, carefully slide a single microarray lengthwise into the tube.
- To remove excess fluid, spin the microscope slide box or the 50 ml tubes containing the microarrays in a centrifuge at 800 rpm for 3 min (spinning at higher speeds may break the microarray). After centrifugation, carefully remove the HuProt™ microarrays and discard the clean room wipes.

**II.10** Scanning:

- The HuProt™ microarrays can be scanned immediately (highly preferred) or stored at -20°C in a lightproof box.

**IMPORTANT:** Microarrays must be scanned within 3 days after the assay is performed.

Appendix for Monoclonal Antibody Specificity Determination:

**Recipes**

- Denaturation Buffer: 9M Urea with 5 mM DTT in water. Dissolve urea in water and filter; add freshly-made DTT to the solution immediately before use.
- 1X TBS-T Buffer: 1X TBS pH 7.5, 0.1% Tween-20 (see General Appendix, Buffers section)
- Blocking Solution: 5% BSA [w/v] in 1X TBS-T Buffer: Dissolve 5 g of Bovine Serum Albumin (IgG-Free, Protease-Free) in 60 ml of 1X TBS-T. Complete to 100 ml with 1X TBS-T and filter before use.

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## III. Serum Profiling Assay

**SERUM SAMPLE STORAGE:** Repeated thawing and re-freezing of serum samples can affect the reproducibility of serum profiling data obtained from Hu¬Prot™ arrays. When collecting serum samples, divide the serum into single-use aliquots and store them at -80˚C. This will optimize the reproducibility of the experiments.

**NOTE:** Typically a 1:1000 dilution of serum is used.

**EPITOPE SPREADING:** Clients who have studied epitope spreading on HuProt™ arrays recommend using a 1:500 dilution of serum.

### III.1 Storage:

- Store HuProt™ microarrays in closed plastic slide holders at -80°C or on a layer of dry ice, right until the blocking step. The active surface of the HuProt™ microarray is the surface with the barcode.

**IMPORTANT:** It is critical to keep the HuProt™ microarrays ultra cold and ultra dry prior to use. Do not let liquid condense onto the microarray surface before use.

### III.2 Blocking:

- Add 3.0 ml of blocking solution (5% BSA/1X TBS-T) to each compartment in the 4-well plates.
- Carefully use fine-nosed tweezers to remove one microarray from the plastic slide holder that has been resting on dry ice. Immediately submerge the HuProt™ microarray, active side up, in a 4-well plate containing blocking buffer. Incubate with gentle shaking for 1.5-2 hrs at room temperature.

### III.3 Sample preparation:

- The recommended serum dilution is 1:1000 in blocking buffer.
- Dilute the primary sample to a final volume of 3.0 ml in blocking buffer, then vortex briefly and store on ice.

### III.4 Assay: Add Sample to HuProt™ Microarray

- Remove the blocking buffer out of a corner of the 4-well plates containing the immersed HuProt™ microarrays by aspiration.

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- Carefully pipette the prepared serum sample (3.0 ml) onto the active surface of the blocked HuProt™ microarray (barcode facing up). Be careful not to let the pipette tip touch the microarray surface.
- Incubate with gentle shaking on an orbital shaker overnight at 4˚C (e.g. in cold room).

**III.5** Washing:

- Following incubation, remove the buffer from the corner of the 4-well plate by aspiration. Add 4.0 ml of 1X TBS-T to each well and rinse briefly. After rinsing, remove the buffer using aspiration.
- Add 4.0 ml of 1X TBS-T and incubate 10 min at room temperature with gentle shaking. Remove the buffer by aspiration from the corner of the 4-well plate. Repeat for a total of three 10 min washes. Do not let the HuProt™ microarray dry out at any time.

**IMPORTANT:** For all steps below, cover the 4-well plates containing the HuProt™ microarrays tightly with aluminum foil during all incubations and washes to minimize light exposure.

**III.6** Detection: Add Secondary Antibodies

- Dilute the secondary antibody in blocking buffer per manufacturer's directions for Western Blot use. Add 3.0 ml of the diluted secondary antibody to each freshly washed HuProt™ microarray. Cover the 4-well plate with aluminum foil and incubate for 1-1.5 hrs with gentle shaking at room temperature.

**III.7** Washing:

- Remove the buffer containing secondary antibodies by aspiration. Add 4.0 ml 1X TBS-T to briefly rinse the microarray. After rinsing, remove the buffer using aspiration.
- Add 4.0 ml of 1X TBS-T and incubate for 10 min at room temperature with gentle shaking. Remove the wash buffer from a corner of the 4-well plate by aspiration. Repeat for a total of three 10 min washes.
- Briefly rinse the microarrays three times with 0.1X TBS.

**IMPORTANT:** Do not let the HuProt™ microarrays sit for long periods in wash buffer. Proceed to the drying stage immediately.

Alternative Final Rinse method:

- Hold the array on the barcoded edge with tweezers. Dip the array rapidly in a fresh beaker of $ddH_20$ three times. Gently blot the edge of the washed array on kimwipes or clean room wipes, and then proceed to the drying step.

**IMPORTANT:** Proceed to the drying stage immediately.

**II.8** Drying:

- Place clean room wipes or paper towels on the bottom of a black microscope slide box (will hold several microarrays) or prepare plastic conical 50-ml tubes (each will hold one microarray).
- Remove the HuProt™ microarray from the 4-well plates and tap the edge lightly on a clean room wipe or paper towel to remove excess fluid. Do not touch the active surface of the microarray. Carefully slot the microarrays into the microscope slide box – the microarrays will be perpendicular to the paper towels lining the box. If you are using 50-ml tubes, carefully slide a single microarray lengthwise into the tube.
- Spin the microscope slide box or the 50-ml tubes containing the microarrays in a centrifuge at 800 rpm for 3 min to remove excess fluid (spinning at higher speeds may break the microarrays). After centrifugation, carefully remove the HuProt™ microarrays and discard the clean room wipe or paper towel.

**III.9** Scanning:

- The HuProt™ microarray can be scanned immediately (highly preferred), or stored at -20°C in a lightproof box.

**IMPORTANT:** Microarrays must be scanned within 3 days after the assay is performed.

Appendix for Serum Profiling Assay:

## Recipes

- 1X TBS-T Buffer: TBS pH 7.5, 0.1% Tween-20 (see General Appendix, buffer recipe)
- Blocking Solution: 5% BSA [w/v] in 1X TBS-T

References:

Hu CJ *et al.* (2012) Identification of new auto-antigens for primary biliary cirrhosis using human proteome microarrays. *Mol Cell Proteomics,* **11(9)**: 669-80.

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## IV. Fluorescently Labeled RNA Binding Assay

### IV.1 Storage:

- Store HuProt™ microarrays in plastic slide holders at -80°C or on a layer of dry ice, right until the blocking step. The active surface of the HuProt™ microarray is the surface with the barcode.

**IMPORTANT:** It is critical to keep the HuProt™ microarrays ultra-cold and ultra-dry before use. Do not let condensate form on the surface before the microarray is used.

### IV.2 Blocking:

- Add 3.0 ml of blocking solution (recipe on Pg. 19) to each compartment of the 4-well plates. Carefully use fine-nosed tweezers to remove one microarray from the plastic slide holder that is resting on dry ice. Immediately submerge the HuProt™ microarray, active surface up, in a compartment of the 4-well plate containing blocking buffer. Incubate at room temperature, with gentle shaking for 1.5-2 hrs.

### IV.3 Sample preparation (low volume assay):

- Dilute the fluorescently labeled RNA sample to 250 nM in 200 µl RNA binding buffer.

### IV.4 Assay: Add Labeled RNA to HuProt™ microarrays

- Remove the HuProt™ microarrays from the 4-well plates and tap the edge lightly on a clean room wipe or paper towel to remove excess buffer.
- Place the microarray in a humidification chamber to prevent evaporation. Carefully pipette the prepared RNA sample onto the active surface of the blocked HuProt™ microarray. Be careful not to let the pipette tip touch the active surface.
- Cover the microarray with a glass cover slip to prevent evaporation.
- Cover the humidification chamber with aluminum foil to minimize light exposure that may quench the fluorescence. Incubate with gentle shaking for 1 hr at room temperature.
  **NOTE:** An empty yellow tip box may be used to create a humidification chamber. Place wet clean room wipes or paper towels in the base of a yellow tip box. Replace the empty plastic tip rack above the base, over the wet clean room wipes or towels. Carefully place up to four cover slipped HuProt™ microarrays on the rack, and close the lid.

**IMPORTANT:** Use a sample volume of at least 200 µl for the assay.

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**IV.5** Washes:

- Add 4.0 ml 1X TBS-T to each compartment in the 4-well plates. Submerge the coverslipped HuProt™ microarray in a compartment and carefully remove the cover slip using fine-nosed tweezers. Be careful not to scratch the active surface of the microarray (alternatively, float off the cover slip by immersing the microarray in a larger volume of wash buffer). Shake gently for 5 min at room temperature, and then remove the wash buffer by aspiration. Repeat for a total of four washes.

**IMPORTANT:** Cover the 4-well plates with aluminum foil at all times to minimize light exposure.

- Briefly rinse the microarrays three times with 0.1X TBS.
- Remove the HuProt™ microarrays from the 4-well plates with tweezers. Tap the edge of the microarray lightly on a clean room wipe to remove excess fluid. Do not touch the active surface of the microarray.

**IV.6** Drying:

- Place clean room wipes or paper towels on the bottom of a black microscope slide box (will hold several microarrays) or prepare plastic conical 50 ml tubes (each will hold one microarray).
- Remove the HuProt™ microarray from the 4-well plates and tap the edge lightly on a clean room wipe to remove excess fluid. Do not touch the active surface of the microarray. Carefully slot the microarrays into the microscope slide box – the microarrays will be perpendicular to the wipes lining the box. If you are using 50 ml tubes, carefully slide a single microarray lengthwise into the tube.
- To remove excess fluid, spin the microscope slide box or the 50 ml tubes containing the microarrays in a centrifuge at 800 rpm for 3 min (spinning at higher speeds may break the microarray). After centrifugation, carefully remove the HuProt™ microarrays and discard the clean room wipes.

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Barry G *et al.* (2013). The long non-coding RNA Gomafu is acutely regulated in response to neuronal activation and involved in schizophrenia-associated alternative splicing. *Molec Psychiatry* **Apr 30.** doi: 10.1038/mp.2013.45. ePub ahead of print.

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Fan B *et al.* (2013). A human microarray identifies that the heterogeneous nuclear ribonucleoprotein K (hd RNP K) recognizes the 5' terminal sequence of the hepatitis C virus RNA. *Mol Cell Proteomics.* **13(1)**:84-92.

**IV.7** Scanning and Storage:

- The HuProt™ microarrays can be scanned immediately (highly preferred), or stored at -20°C in a lightproof box.

**IMPORTANT:** Microarrays must be scanned within 3 days after the assay

Appendix for Fluorescently-labeled RNA-binding Assay

**Recipes**

- 1x TBS-T (See General Appendix)
- Blocking Solution: 10 µg/ml ssDNA (herring sperm); 2 mM $MgCl_2$; 5 mg/ml BSA in SuperBlock T20 (PBS) Blocking Buffer (Thermo Scientific Cat. No. 37516)
- RNA Binding Buffer: 2 mM $MgCl_2$; 2 mg/ml BSA; 10 µg/ml ssDNA (herring sperm) in 1X PBS

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## Assays Using the HuProt™ Human Proteome Microarray

### V. Fluorescently Labeled DNA Binding Assay

**V.1** Storage:

- Store HuProt™ microarrays in closed plastic slide holders at -80°C or on a layer of dry ice, right until the blocking step. The active surface of the HuProt™ microarray is the surface with the barcode.

**IMPORTANT:** It is critical to keep the HuProt™ microarrays ultra-cold and ultra-dry before use. Do not let condensate form on the surface before the microarray is used.

**V.2** Blocking:

- Place the HuProt™ microarrays in a humidification chamber.
- Carefully pipette 200 µl of DNA hybridization buffer (pre-chilled on ice) onto the active surface of each HuProt™ microarray. Do not let the pipette tip touch the microarray surface. Cover the microarray with a glass cover slip to prevent evaporation. Cover the humidification chamber with aluminum foil to minimize exposure to light. Incubate with gentle shaking for 3 hrs at 4°C.

**NOTE:** An empty yellow tip box may be used to create a humidification chamber. Place wet clean room wipes or paper towels in the base of a yellow tip box. Replace the empty plastic tip rack above the base, over the wet clean room wipes or towels. Carefully place up to four cover slipped HuProt™ microarrays on the rack, and close the lid.

**V.3** Sample Preparation:

- (Low volume assay) Dilute the fluorescently labeled DNA sample to 40 nM in 200 µl of DNA hybridization buffer containing poly (dA-dT).

**V.4** Assay: Add Labeled DNA to the HuProt™ microarrays

- After blocking, the cover slip must be removed. Immerse the blocked microarray in 4.0 ml of pre-chilled wash buffer and carefully remove the cover slip using fine-nosed tweezers, being careful not to scratch the active surface of the microarray (alternatively, float the cover slip off by immersing the microarray in a larger volume of wash buffer). Drain off residual buffer by tapping the microarray sideways on clean room wipes.

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- Place the HuProt™ microarrays back in the humidification chamber. Immediately pipette the fluorescently labeled DNA sample (200 µl) onto the active surface of the HuProt™ microarray. Do not let the pipette tip touch the microarray surface.
- Cover the microarray with a glass cover slip to minimize evaporation.
- Cover the humidification chamber with aluminum foil to minimize exposure to light. Incubate with gentle shaking overnight at 4°C.

**V.5** Washing:

- One wash cycle is sufficient for this assay. Immerse the coverslipped microarray in 4.0 ml of pre-chilled wash buffer in a compartment of a 4-well plate. Carefully remove the cover slip using fine-nosed tweezers, taking care not to scratch the active surface (alternatively, float the cover slip off by immersing the microarray in a larger volume of wash buffer). Incubate with gentle shaking for 1-3 min at at 4°C.
- Remove the HuProt™ microarrays from the wash buffer and tap the edge lightly on a clean room wipe or paper towel to remove excess buffer. Do not touch the active surface of the microarray.

**V.6** Drying:

- Place clean room wipes or paper towels on the bottom of a black microscope slide box (will hold several microarrays) or prepare plastic conical 50-ml tubes (each will hold one microarray).
- Remove the HuProt™ microarray from the 4-well plates and tap the edge lightly on a clean room wipe to remove excess fluid. Do not touch the active surface of the microarray. Carefully slot the microarrays into the microscope slide box, placing the microarrays perpendicular to the paper towels lining the base of the box. If you are using 50-ml tubes, carefully slide a single microarray lengthwise into the tube.
- Spin the microscope slide box or the 50-ml tubes containing the microarrays in a centrifuge at 800 rpm for 3 min to remove excess fluid (spinning at higher speeds may break the microarray). After centrifugation, carefully remove the HuProt™ microarrays and discard the clean room wipes.

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Reference:

Hu S *et al.* (2009) Profiling the human protein-DNA interactome reveals ERK2 as a transcriptional repressor of interferon signaling. *Cell* **139(3):** 610-22.

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**V.7** Scanning and Storage:

- The HuProt™ microarrays can be scanned immediately (preferred), or stored at -20°C in a lightproof box.

**IMPORTANT:** Microarrays must be scanned within 3 days after the assay is performed.

Appendix for Fluorescently Labeled DNA Binding Assay

## Recipes

- DNA hybridization buffer (store on ice or at 4˚C): 25 mM HEPES at pH 8.0; 50 mM potassium glutamate; 0.1% Triton X-100; 8 mM magnesium acetate; 3 mM DTT (freshly made and added to the buffer right before use); 4 µM poly (dA-dT); 10% glycerol
- Wash buffer: Same as above, but without 4 µM poly (dA-dT)

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## VI. Fluorescently Labeled Protein Binding Assay

**VI.1** Storage:

- Store HuProt™ microarrays in closed plastic slide holders at -80°C or on a layer of dry ice, right until the blocking step. The active surface of the HuProt™ microarray is the surface with the barcode.

**IMPORTANT:** It is critical to keep the HuProt™ microarrays ultra-cold and ultra-dry before use. Do not let condensate form on the surface before the microarray is used.

**VI.2** Blocking:

- Add 3.0 ml of blocking solution (5% BSA in 1X TBS-T) to each compartment of the 4-well plates. Carefully use fine-nosed tweezers to remove one microarray from the plastic slide holder that is resting on dry ice. Immediately submerge the HuProt™ microarray, active surface up, in a compartment of the 4-well plate containing blocking buffer. Incubate at room temperature with gentle shaking for 1.5-2 hrs.

**VI.3** Sample Preparation:

- **A.** High volume assay: Dilute 3 µg of the fluorescently labeled sample protein to be tested in 3.0 ml blocking solution.
- **B.** Low volume assay: If a limited amount of sample protein is available for testing, dilute 200 ng of labeled sample protein in 200 µl of blocking solution.

**VI.4** Assay: Add Labeled Protein to HuProt™ micorarrays

- (High volume assay): Add 3.0 ml of diluted labeled protein (3 µg protein in 3.0 ml blocking solution) to a 4-well plate. Immerse one blocked HuProt™ microarray into each compartment with the active side up. Cover the plates with aluminum foil to minimize light exposure, which may quench the fluorescence. Incubate with gentle shaking for 1 hr at room temperature.
- (Low volume assay): If a limited amount of labeled protein is available, carefully pipette the diluted sample (200 ng protein in 200 µl blocking solution) onto the active surface of the blocked HuProt™ microarray. Be careful not to let the pipette tip touch the microarray surface. Cover the

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microarray with a fresh cover slip. Place the coverslipped microarrays in a humidification chamber to minimize evaporation. Cover the humidification chamber with aluminum foil to minimize light exposure. Incubate with gentle shaking at room temperature for 1 hr.

**NOTE:** An empty yellow tip box may be used to create a humidification chamber. Place wet clean room wipes or paper towels in the base of a yellow tip box. Replace the empty plastic tip rack above the base, over the wet clean room wipes or towels. Carefully place up to four cover slipped HuProt™ microarrays on the rack, and close the lid.

- Cover the humidification chamber with aluminum foil to minimize light exposure, and incubate with gentle shaking at room temperature for 1 hr.

**VI.5** Washing:

- **A.** High volume assay (reactions using 3.0 ml of diluted sample): Remove the reaction solution from a corner of the 4-well plates by aspiration. Add 4.0 ml 1X TBS-T to briefly rinse the microarray. After rinsing, remove the buffer using aspiration.
- Add 4.0 ml 1X TBS-T buffer and wash with gentle shaking for 10 minutes. Repeat for a total of three long washes.
- **B.** Low volume assay (reactions using 200 µl of diluted sample): Add 4.0 ml 1X TBS-T buffer to each compartment of a 4-well plate. Submerge the cover-slipped HuProt™ microarray in a compartment, and carefully remove the cover slip using fine-nosed tweezers — do not touch the active surface of the microarray. Alternatively, float off the cover slip by immersing the covered microarray in a large volume of wash buffer. Briefly rinse the microarray, and then remove the buffer by aspiration.
- Add 4.0 ml 1X TBS-T buffer and wash with gentle shaking for 10 minutes. Repeat for a total of three long washes.

**IMPORTANT:** Cover the 4-well plates with aluminum foil at all times during both the reaction and washing steps to minimize light exposure, which could quench the fluorescence of your protein sample.

- Briefly rinse the microarrays three times with 0.1X TBS.

**VI.6** Drying:

- Place clean room wipes or paper towels on the bottom of a black microscope slide box (will hold several microarrays) or prepare plastic conical 50-ml tubes (each will hold one microarray).
- Remove the HuProt™ microarray from the 4-well plate and tap the edge lightly on a paper towel to remove excess fluid. Do not touch the active sur-

face of the microarray. Carefully slot the microarrays into the microscope slide box - the microarrays will be perpendicular to the paper towels lining the box. If you are using conical plastic 50-ml tubes, carefully slide a single microarray lengthwise into the tube.

- To remove excess fluid, spin the microscope slide box or the 50-ml tubes containing the microarrays in a centrifuge at 800 rpm for 3 min (spinning at higher speeds may break the microarray). After centrifugation, carefully remove the HuProt™ microarrays and discard the clean room wipe.

**VI.7** Scanning and Storage:

- The HuProt™ microarrays can be scanned immediately (highly preferred) or stored at -20°C in a lightproof box.

**IMPORTANT:** Microarrays must be scanned within 3 days after the assay is performed.

Appendix for protein binding assay using fluorescently-labeled protein probes

## Recipes

- 1X TBS-T (see General Appendix)
- Blocking solution (5% BSA in 1X TBS-T)

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Huang Y *et al.* (2012). Global tumor protein p53/p63 Interactome: making a case for cisplatin chemoresistance. *Cell Cycle* **11(12):**2367-79.

Chen Y. *et al.* (2013). Bcl2-associated Athanogene 4 Interactive Analysis Reveals a New Role in Modulating Proteosome Activity. *Mol Cell Proteomics* **12(10):**2804-19.

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# General Appendix

## Buffers:

- TBS Buffer Recipe (1X):
  - 20 mM Tris-Cl, pH 7.5
  - 150 mM NaCl
- 10X TBS:
  - For 1 liter of 10X TBS stock buffer, dissolve the following in 800 ml of distilled water:
    - 24.0 g Tris
    - 87.6 g NaCl
    - Adjust pH to 7.5 and add distilled water to 1L. Sterilize by autoclaving or by filtration, and store at room temperature.
- TBS-T (1X):
  - Add 1.0 ml Tween-20 to 100 ml 10X TBS solution. Make up to 1 liter with distilled water. Stir until homogenous (Tween-20 is very viscous and may stick to pipette tips. A 10% solution is easier to dispense than the undiluted form).
- Phosphate Buffered Saline (PBS) Recipe (1X):
  - 137 mM NaCl
  - 2.7 mM KCl
  - 10 mM $Na_2HPO_4 \cdot 2H_2O$
  - 1.8 mM $KH_2PO_4$
- Blocking Solution Recipes:

  **NOTE:** Blocking conditions vary depending on the protocol used. Please refer to each section for the correct recipes.

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# General Appendix

## HuProt™ Human Proteome Microarray Literature Citations


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