CDI Laboratories

User Guide

HuProt™ Human Proteome Microarray v2.0

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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
Slide type	Coated glass*
Detection method	Fluorescence
Number of proteins	19,394

*The HuProt™ human proteome microarray is available on a variety of glass surfaces including FAST™, FullMoon™, SuperEpoxy™, SuperAldehyde™, SuperNHS™, Ni-NTA, PATH, and Schott.

The HuProt™ version 2.0 microarray contains 19,394 unique proteins (19,275 individually purified human and 119 mouse proteins). This content encompasses 15,275 unique human genes and 119 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:

- **H1** - Histone H1
- **H2 (A+B)** - Histone H2A and H2B mixture
- **H3** - Histone H3
- **H4** - Histone H4 (all these histones are non-specific binding proteins used as positive controls for all kinds of assays.)
- **IgG488/594** - Alexa Fluor 488/594 labeled IgG, positive control and landmarks for fluorescent detection in 488/594 channels.
- **IgG555/647** - Alexa Fluor 555/647 labeled IgG, positive control and landmarks for fluorescent detection in 555/647 channels.
- **GST10n** - glutathione S-transferase at 10 ng/µl
- **GST50n** - GST at 50 ng/µl
- **GST100n** - GST at 100 ng/µl
- **GST200n** - GST at 200 ng/µl
- **Mouse-anti-biotin**
- **Rabbit-anti-biotin**
- **Biotin-BSA** - biotinylated BSA
- **BSA** - Bovine serum albumin, negative control
- **Buffer** - printing buffer only, negative control
- **Mouse IgM**

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Summary, continued from page 1.

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 3D polymers that contain functional groups. This allows the protein samples to be immobilized on the glass by covalent bonding.

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.
- When conducting low volume assays, be very careful when adding glass cover slips to the active surface of the microarray (used to minimize evaporation). Likewise, when the assay is completed, be careful when removing the cover slips from the microarrays prior to the washing steps. If the microarray surface is scratched, proteins printed on the glass may be smudged or removed. One alternative is to immerse the covered microarray in a large volume of wash buffer, and then allow the cover slip to 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 from storage, place a layer of dry ice pellets inside a styrofoam box with a lid and cover tightly. Remove a plastic slide holder containing HuProt™ microarrays from storage at -80°C 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 holder.

Prepare 4-well plates for the blocking step

- 4-well plates are used to hold the HuProt™ microarrays during the blocking, reaction (in the case of high volume samples) and washing steps (e.g. * Nunc* 4-well rectangular dishes, Fisher Scientific No.12-565-495). Each compartment of the 4-well plates is used to hold one microarray. To minimize cross-contamination of samples during the reaction step, some users choose to keep one compartment empty in between 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. Be careful 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. Carefully pour off the blocking buffer or remove it by aspiration. Add 3.0 ml fresh blocking buffer to the well of the plate and incubate as directed in the protocol.
- 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 protocol of interest for the correct recipe.

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Sample Preparation and Assay

- High volume assays: If a high volume of sample is available to test, dilute it in 3.0 ml of buffer. Add this to one compartment of a 4-well plate. Immerse the HuProt™ microarray face up in the 3.0 ml of diluted test sample (no coverslip is needed).
- For low volume assays (100 - 300 µl), evaporation must be minimized. Add the 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: If you do not have a humidification chamber, an empty yellow tip box may be adapted to this purpose. Place wet clean room wipes or paper towels in the base of the yellow tip box and replace the empty tip rack above them. Carefully place up to four coverslipped HuProt™ microarrays on the rack, and then close the lid.

Washing

- When assays are completed, the microarrays are washed in 4-well plates. For low volume reactions carried out in a humidification chamber, the cover slip must first be removed without touching or scratching the microarray surface. Immerse the covered microarray in a compartment of a 4-well plate into which 4.0 ml of wash buffer has previously been added (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. Do not touch or scratch the active surface of the microarray. An alternative is to float the cover slip off by immersing the microarray in a larger volume of wash buffer.
- For high volume samples, the washes may be carried out in the same 4-well plates in which assays and blocking were conducted.

IMPORTANT: If fluorescent probes are used at any point, light exposure, which may quench fluorescence, must be minimized. 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 assays 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 varies 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. Carry out anti-GST probing after the primary assay has been conducted on the microarray and the data scanned, using an anti-Glutathione-S-Transferase antibody (e.g. Glutathione-S-Transferase, *S. japonicum* form; EMD Millipore, cat no. A-21428). Next, select 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 or Fisher Scientific No.12-565-495)

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

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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 prior to use. Do not let liquid condense onto the microarray surface before use.

I.2 Blocking:

- Add 3.0 ml of blocking solution (5% BSA/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 with gentle shaking for 5 min. Carefully pour off the blocking buffer or remove it by aspiration. Add 3.0 ml fresh blocking buffer to the well of the plate and incubate the microarray at room temperature for 1.5-2 hrs with gentle shaking.

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

- (Low volume reaction): If the primary antibody to test is available in a limited amount, dilute the primary antibody to a concentration of 400 ng/ml in 300 µl blocking buffer. Store on ice.
- (High volume reaction): If the amount of primary antibody to be tested is not limiting, dilute the primary antibody to a concentration of 400 ng/ml in 3.0 ml blocking buffer. Store on ice.
- If the antibody source is in supernatant form:

  Make a 1:12 dilution of the supernatant in blocking buffer, diluted to a final volume of 300 µl. Store on ice. **NOTE:** This dilution assumes that the antibody concentration is 0.005 mg/ml of supernatant. 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.
- 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.

IMPORTANT: Use a sample of at least 100 µl.

- 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 TBS-T, completely removing the buffer after each wash by aspiration. Repeat for a total of three short washes.
- Add 4.0 ml of 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.

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.

- 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.

I.7 Washing:

- Add 4.0 ml 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 slides three times with ddH_2O.

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 after the assay is performed.

Appendix for Monoclonal Antibody Specificity Determination:

Recipes

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

Assays Using the HuProt™ Human Proteome Microarray

II. Serum Profiling Assay

NOTE: Typically a 1:500 dilution of serum in blocking buffer is used.

II.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.

II.2 Blocking:

- Add 3.0 ml of blocking solution (5% BSA/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 5 min. Carefully pour off the blocking buffer or remove it by aspiration. Add 3.0 ml fresh blocking buffer to the well of the plate and incubate the microarray with gentle shaking for 1.5-2 hrs at room temperature.

II.3 Sample preparation:

- The recommended serum dilution is 1:500 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.

II.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.
- 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 for 1 hr at room temperature.

II.5 Washing:

- Following incubation, remove the buffer from the corner of the 4-well plate by aspiration. Add 4.0 ml of TBS-T to each well and rinse briefly. Repeat for a total of three short washes.
- Add 4.0 ml of 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.

II.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.

II.7 Washing:

- Remove the buffer containing secondary antibodies by aspiration. Add 4.0 ml TBS-T to briefly rinse the microarray; repeat for a total of three short rinses.
- Add 4.0 ml of 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 slides three times with ddH_2O.

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).

Reference:

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

- 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.

II.9 Scanning:

- The HuProt™ microarray can be scanned immediately (highly preferred), or it can be 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

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

Assays Using the HuProt™ Human Proteome Microarray

III. Fluorescently Labeled RNA Binding Assay

III.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 prior to use. Do not let liquid condense onto the microarray surface before use.

III.2 Blocking:

- Add 3.0 ml of blocking solution to each compartment in 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 side up, in a compartment containing blocking buffer. Incubate with gentle shaking for 5 min. Carefully pour off the blocking buffer or remove it by aspiration. Add 3.0 ml fresh blocking buffer to the compartment in the 4-well plate and incubate the microarray with gentle shaking for 1.5-2 hrs at room temperature.

III.3 Sample preparation (low volume assay):

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

III.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.

- If you do not have a humidification chamber, you can adapt an empty yellow tip box for this purpose. Place wet clean room wipes or paper towels in the base of the yellow tip box and replace the empty tip rack on top of the towels. Carefully place up to four coverslipped HuProt™ microarrays on top of the rack, and then close the lid to create a humid environment.

IMPORTANT: Use a sample volume of at least 100 µl for the assay.

III.5 Washes:

- Add 4.0 ml PBS-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 three washes.

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

- Briefly rinse the microarrays three times with ddH_2O.
- 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.

III.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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III.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 Fluorescently-labeled RNA-binding Assay

Recipes

- PBS-T (see General Appendix, Recipe 2)
- 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

References:

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.

Donnelly CJ *et al.* (2013). RNA Toxicity from the ALS/FTD C90RF72 Expansion is Mitigated by Antisense Intervention. *Neuron* **80(2):** 415-28.

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. Fluorescently Labeled DNA Binding Assay

IV.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 in a cold, dry environment, and to prevent liquid from condensing onto the microarray surface before use.

IV.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: If you do not have a humidification chamber, you can adapt an empty yellow tip box for this purpose. Place wet clean room wipes or paper towels at the base of the yellow tip box and replace the empty tip rack above the towels. Carefully place up to four coverslipped HuProt™ microarrays on the rack, and then close the lid.

IV.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).

IV.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.

- 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.

IV.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.

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, 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.

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.

IV.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 added to buffer before use); 4 µM poly (dA-dT); 10% glycerol
- Wash buffer: Same as above, but without 4 µM poly (dA-dT)

V. Fluorescently Labeled Protein 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, and to prevent liquid from condensing onto the microarray surface before use.

V.2 Blocking:

- Add 3.0 ml of blocking solution (5% BSA in PBS-T) to each compartment of a 4-well plate. Carefully use fine-nosed tweezers to remove one microarray from the plastic slide holder resting on dry ice. Immediately submerge the HuProt™ microarray, active side up, in a compartment containing blocking solution. Incubate with gentle shaking for 5 min. Carefully pour off the blocking buffer or remove it by aspiration. Add 3.0 ml fresh blocking buffer to the well of the plate and incubate the microarray with gentle shaking for 1.5-2 hrs at room temperature.

V.3 Sample Preparation:

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

V.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 microarray with a fresh cover slip. Place the coverslipped microarrays in a humidification chamber to minimize evaporation.

NOTE: If you do not have a humidification chamber, you can adapt an empty yellow tip box for this purpose. Place clean room wipes or wet paper towels at the base of the yellow tip box and replace the empty tip rack on top of the towels. Carefully place up to four coverslipped HuProt™ microarrays on the rack, and then 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.

V.5 Washing:

- (High volume assay): For reactions using 3.0 ml of diluted test protein, remove the reaction buffer from a corner of the 4-well plate by aspiration. Add 4.0 ml PBS-T to briefly rinse the microarray, and remove the buffer by aspiration. Repeat for a total of three short washes.
- (Low volume assay): For reactions using a low volume of diluted test protein (200 µl), add 4.0 ml PBS-T to each compartment of a 4-well plate. Submerge the coverslipped HuProt™ microarray in a compartment and carefully remove the cover slip using fine-nosed tweezers, being careful not to touch the active surface of the microarray (alternatively, float the cover slip off by immersing the microarray in a larger volume of wash buffer). Briefly rinse the microarray, and then remove the buffer by aspiration. Repeat for a total of three short washes in PBS-T.

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 ddH_2O.

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 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.

V.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

- PBS-T (see General Appendix Buffer Recipe)
- Blocking solution (5% BSA in PBS-T)

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Buffers:

- TBS Buffer Recipe (1X):
  - 50 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:
    - 60.5 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:
  - 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 $Na_2HPO_4 \cdot 2\ H_2O$
  - 1.8 mM KH_2PO_4
- 10X PBS
  - For 1 liter of 10X PBS stock buffer, dissolve the following in 800 ml distilled water:
    - 80.0 g NaCl
    - 2.0 g KCl
    - 14.4 g mM $Na_2HPO_4 \cdot 2\ H_2O$
    - 2.4 g KH_2PO_4
    - Adjust pH to 7.4 and add distilled water to 1 liter. Sterilize by autoclaving or filtration. Store at room temperature.
- PBS-T
  - Add 1.0 ml Tween-20 to 100 ml 10X PBS 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).
- 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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