HuProt™ Human Proteome Microarray v4.0
—User Manual—

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Microarray Type	**Functional Protein Microarray**
Species	Human
Production technology	Non-contact piezoelectric inkjet
Slide type	Coated glass (PATH®)
Detection method	Fluorescence
Content	Recombinant human & mouse proteins[a]

[a] Please refer to the documentation accompanying the microarrays for specific information.

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1 Technology Overview

CDI Labs' HuProt™ Human Proteome Microarray is the most comprehensive human full-length protein array yet created (Jeong et al, 2012). Our latest version, HuProt™ v4.0, contains over 21,000 human proteins and protein isoforms. This covers over 81% of human proteins in each major functional category of the proteome, as defined by the Human Protein Atlas (Venkataraman et al, 2018), and allows hundreds of interactions to be profiled at once in a wide range of applications, including mapping antigen-specific immunity (multi-isotype serum profiling), determining monoclonal antibody specificity, and facilitating studies of protein-protein interaction, substrate identification, protein-DNA binding, protein-RNA binding, and the binding of some small molecules.

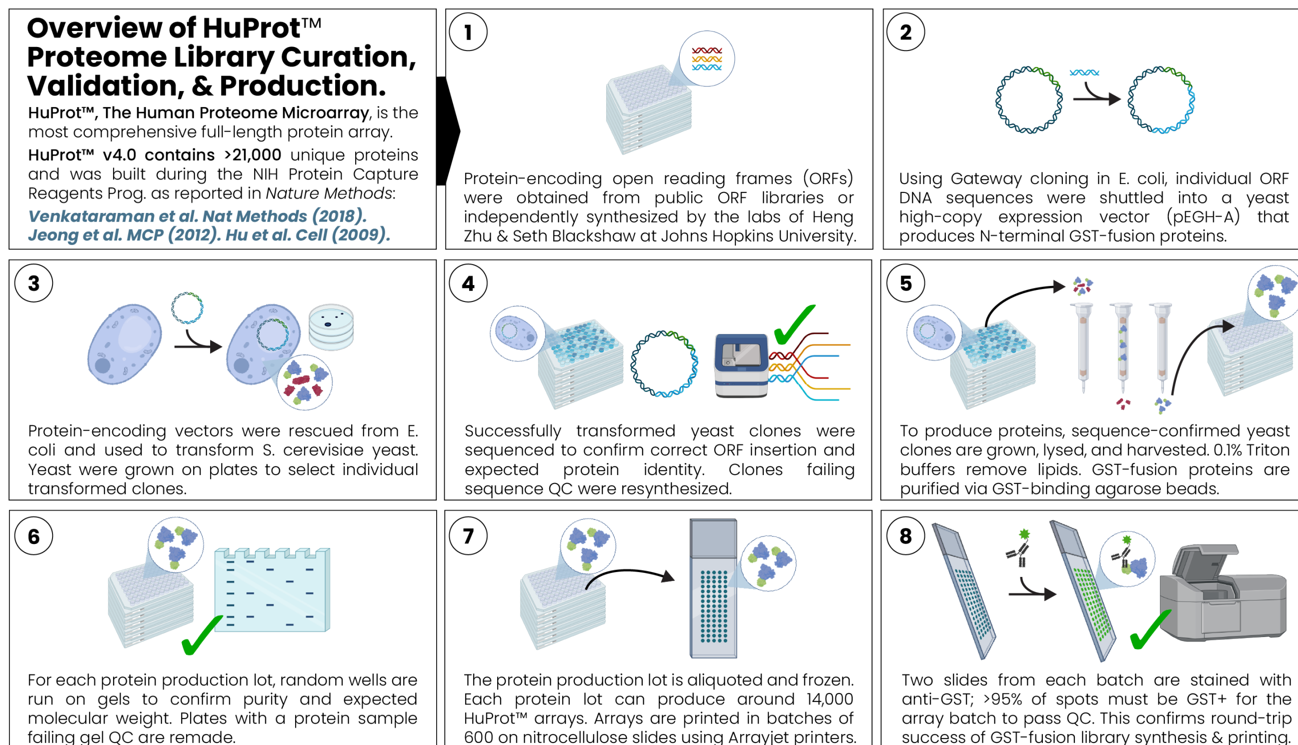


Figure by Tyler Hulett, PhD for CDI Antygen; created with BioRender.

The HuProt™ expression library was created by inserting full-length human open reading frames (ORFs) into a yeast high-copy expression vector that produces GST-His6 fusion proteins when induced in yeast (Hu S et al 2009; Jeong J et al 2012).

Each human ORF expression vector was verified for integrity by Sanger sequencing (Venkataraman et al, 2018). These sequence-verified expression vectors encoding human ORFs were transformed into yeast to create our master library; this eukaryotic expression system ensures that HuProt™ proteins maintain both function and proper conformation (Hu S, poster at https://cdi.bio/wp-content/uploads/2019/11/CDI_Well_Folded_Protein_Poster.pdf).

As required — about once per year — this yeast library is used to synthesize human proteins in high-throughput. Full-length synthesized proteins are purified via their GST-His6 fusion tags as previously described (Hu S et al, 2009). These proteins are then frozen in aliquots in a 384-well format for later use in printing on HuProt™ microarrays.

To print individual batches of HuProt™ arrays, the purified human proteins are then printed as duplicate spot pairs using an Arrayjet UltraMarathon printer (Arrayjet, UK) on PATH nitrocellulose slides (GraceBio, USA). Controls on HuProt™ include titrated GST protein, titrated human IgG, other human antibody isotypes, histones, mouse and rabbit anti-biotin, mouse IgM, and biotin-tagged control for streptavidin detection. HuProt™ also contains landmark control spots, including Alex Fluor 555/647 (Jeong et al, 2012). Success of each batch of HuProt™ microarrays is validated by anti-GST staining. This confirms that full-length GST-His6 fusion proteins were successfully expressed, synthesized, purified, and printed across our clone collection.

2 Control Spots

Control	Function
H1 - Histone H1 H2 (A+B) - Histone H2A and H2B mixture H3 - Histone H3 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 fluorescence detection in 488/594 channels.
Rhodamine + IgG 647	Rhodamine + Alexa Fluor 647 labeled IgG, a positive control and landmarks for fluorescence 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 Human IgG 6.25 ng/μL Human IgG 25 ng/μL 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 Anti-Human IgG 6.25 ng/μL Anti-Human IgG 25 ng/μL 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 GST (glutathione S-transferase) 50 ng/μL GST (glutathione S-transferase) 100 ng/μL 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.

NOTE: The active surface of the HuProt™ microarray is also the barcoded side. The microarrays should only be handled at the barcoded end of the microarray. Wear gloves and use tweezers to handle the microarrays.

3 Storage and Handling

3.1 Storage

HuProt™ microarrays are shipped in closed plastic slide holders on dry ice. Please store the microarrays at -80℃ immediately upon receipt; be sure to keep the slide holders closed. Each provided holder contains up to x5 HuProt™ arrays.

3.2 Handling

• Always wear gloves

• Do not touch the active surface of the microarray that the proteins are printed on. This is the same surface with the barcode; the barcode also marks the end of the microarray.

• When handing, use tweezers to handle the barcoded end of the microarray.

• The active surface of the microarray must always face up during assays and rinses.

• Do not let the microarray dry out at any time during the procedures.

3.3 Additional Materials and Instruments for Assays

Aluminum foil
Cleanroom wipes (preferred) or paper towels
Micropipettes
Orbital shaker
Sterile disposable micropipette tips
Sterile serological pipettes (25 mL)
Fine-nosed tweezers
Vacuum system
Plastic rectangular 4-well plates with lids (e.g. Thermo Scientific™ Nunc™ Dishes, Rectangular 4-Well, No.12-565-495)
Lightproof Microscope Slide Box
1000mL or 250mL Filter Units (PES membrane, 0.45 μm)
1000mL or 250mL Receiver Units
15.0 mL plastic screw-top tubes
Micropipette tips
Nitrile gloves
Compressed air duster

3.4 Additional Equipment for Data Analysis

Microarray scanner (e.g. Molecular Devices GenePix 4000B)
Microarray data analysis program (e.g. GenePix Pro 6.1)

4 Data Processing and Analysis Rescources

4.1 Raw Data Extraction From the .gal File

After scanning, HuProt™ microarrays must be aligned to a file of the array layout (.gal), in order to extract the raw microarray data (.gpr). The most up to date .gal file (ie: HuProt_v4.0_Standard_Gal_Updated_01212022.gal) is always available on this webpage:

https://collection.cdi-lab.com/public/downloads

The above .gal file works with common microarray scanning systems such as Molecular Devices Genepix and Innopsys Innoscan Mapix. Please confirm with your microarray scanner manufacturer that HuProt™ is compatible with your systems. More information on .gal files (genepix layout), and .gpr (genepix results) can be found on on the Molecular Devices website:

https://support.moleculardevices.com/s/article/GenePix-File-Formats

4.2 Initial Data Processing From the .gpr Files

A variety of online tools and resources exist to support microarray data extraction, normalization, and analysis from .gpr files (ie Limma, PAWER, etc). However, not all of the tools and features of these programs are necessarily helpful or appropriate for protein microarray analysis. As yet, there is no scholarly consensus on 'the best way' to analyze HuProt™ data, primarily due to the versatility and variety of studies and assays which can be done with protein microarrays. Different assays and study goals require variation in hit extraction, raw data normalization, etc.

Spots labeled ND (no data) did not pass our collection sequencing processing QC steps as reported (Venkataraman et al. 2018); corrected versions are printed elsewhere on the array. Every protein is printed 'as-purified' and varies in concentration. Parent protein aliquots are known to range from 0.001-2.5 mg/mL in concentration, with an average of 0.15 mg/mL. Each protein aliquot is printed in duplicate with 100 picoliters (pL) volume per protein spot.

In order to support you in processing your .gpr files, a simple script is available here to process a folder of .gpr files into 'stitched output tables:'

https://github.com/cdi-lab/StarterKit

The above is a set of simple R scripts for basic, stand-

alone analysis of HuProt™ (gpr) result files. Our data processing StarterKit uses foreground median fluorescence intensity values from each spot pair and averages them; it then outputs a 'raw' and 'quantile normalized' set of files.

IMPORTANT: We do not recommend subtracting the spot-specific background as this is typically both very low on properly stained arrays and can contain 'real signal' from the array spot itself. The nitrocellulose surface most HuProt™ arrays are printed on has a limited protein absorbance capacity, and higher concentration spots will often bleed over and result in 'real signals' in the background halo captured around each spot by GenePix and Mapix software.

4.3 Additional Content and Analysis Resources

The most up to date files related to HuProt™ content and data analysis are always available at this website. File names will be as below, or similar:

https://collection.cdi-lab.com/public/downloads

HuProt_V4.0.zip: This contains the complete amino acid sequence of every protein in the HuProt™ collection. The HuProt™ v4.0 zip file includes these columns: system ID, symbol, nucleotide, protein, reviewed UniProt, unreviewed UnProt, CCDS, RefSeq, Ensembl, synonyms, categories.

230414_-_HuProt_HPA_Alignment.xlsx: This contains the Human Protein Atlas alignment with HuProt™ and is used for ontology enrichment analysis, subcellular localization information, etc. The nomenclature alignment is done using a new BLAST results file (230414 - HuProt BLAST Nomenclature.xlsx).

230414_-_HuProt_BLAST_Nomenclature_.xlsx: This contains the HuProt™ Sequence BLAST Label Curation versus UniProt (Human and Mouse). No sequences are altered; only the gene symbols are updated.

5 General Protocol

This protocol uses CDIArrayBlock™ and/or Seromics™ Sample Buffer which are included with each HuProt™ v4.0 microarray kit. The following general protocol is used for the assays of:

- **Serum (or plasma) samples**
- **Antibody samples**
- **Biotinylated protein samples**
- **Biotinylated small molecules**

IMPORTANT: Sample handling and dilution descriptions are modular; the core assay is identical across these sample types.

If you have questions about testing non-biotinylated proteins and small molecules, please contact info@cdi.bio.

5.1 General Reagents

Bovine Serum Albumin (IgG-Free, Protease-Free) (e.g. Jackson ImmunoResearch Laboratories 001-000-173)
10X TBS (Tris buffered saline)
Tween® 20
ddH2O

5.2 General Recipes

• **1X TBS-T Buffer (also used as a Washing Buffer):** TBS pH 7.4, 0.1% Tween™ 20. Commercially available 10X TBS may also be purchased. Store the buffer at room temperature.

• **Detection Buffer:** 5% BSA in 1X TBS-T. Filter the solution using a 0.45 μm filtration system, and store at 4℃ until use.

IMPORTANT: Two tubes of buffer are included with the HuProt v4.0 microarrays. **Do not use these buffers if you are testing DNA or RNA binding on HuProt™ microarrays.** Please refer to the CDI User Manual for the relevant protocols for testing nucleic acids.

CDIArrayBlock™ (provided by CDI Labs in 3.0 mL aliquots; store at 4℃). This can be used for serum, antibody, protein, and small molecule samples.

Seromics™ Sample Buffer (provided by CDI Labs in 3.0 mL aliquots; store at 4℃; **for serum/plasma samples only**)

5.3 Sample Preparation:

Prepare your samples of interest according to their type below.

5.3.1 Serum (or Plasma) Sample Preparation

1. Thaw the frozen serum (or plasma) samples and place on ice.
2. Remove the tubes of CDIArrayBlock™ and Seromics™ Sample Buffer from storage at 4℃ and place on ice (one tube of each buffer is needed for each HuProt™ microarray).
3. Label the Seromics™ Sample Buffer tubes with the sample names.
4. Add 3.0 µL of neat serum (or plasma) to the Seromics™ Sample Buffer and invert to mix.
5. Incubate at room temperature for 1 hour with gentle shaking. This incubation step can be done when the HuProt™ microarrays are being blocked (section 5.5).

5.3.2 Antibody Specificity Sample Preparation

1. Remove the tubes of CDIArrayBlock™ buffer from storage at 4℃ and place on ice (one tube of the buffer is needed for each HuProt™ microarray).
2. Thaw the monoclonal or polyclonal antibody samples and place on ice until ready to use. Each array uses 3 µg of antibody. The recommended antibody stock concentration is at least 0.1 mg/mL.

5.3.3 Biotinylated Protein Sample Preparation

1. Remove the tubes of CDIArrayBlock™ buffer from storage at 4℃ and place on ice (one tube of the buffer is needed for each HuProt™ microarray).
2. Thaw the biotinylated protein samples and store on ice until ready to use. Each array uses 3 µg of protein sample. The recommended protein stock concentration is at least 0.1 mg/mL.

   If you have question about protein samples with other labels, please contact us at info@cdi-lab.com.

5.3.4 Biotinylated Small Molecule Sample Preparation

1. Remove the tubes of CDIArrayBlock™ from storage at 4℃ and place on ice (one tube of the buffer is needed for each HuProt™ microarray).
2. Prepare your small molecule samples at a concentration of 1 mM. Each array uses 30 µL of small molecule sample.

5.4 HuProt™ Microarray Preparation

1. Set aside the exact number of rectangular 4-well plates needed and label them with a permanent marker.
2. Remove plastic slide holders containing HuProt™ microarrays from -80℃ storage; keep the plastic slide holders flat on a layer of dry ice until the blocking stage.

IMPORTANT: Purified proteins are printed on the side of the HuProt™ microarray with the barcode to create the "active surface." The barcode marks the end of the microarray.

IMPORTANT: Keep the HuProt™ microarrays ultra cold/ultra dry before blocking. Do not let condensation form on the microarray surface.

5.5 Block the HuProt™ Microarrays

1. Use a pipette to add 3.0 mL of CDIArrayBlock™ buffer to each well in the rectangular 4-well plates. Each well will hold one HuProt™ microarray for the assays and subsequent steps.

   NOTE: Some users leave an unused, empty well in between each microarray to minimize cross contamination.

2. After all the wells are filled with CDIArrayBlock™ buffer, remove the plastic slide holders containing the HuProt™ arrays from the -80℃ freezer, and place the holders flat on a layer of dry ice. The barcodes on the microarrays should be facing up.

3. Carefully remove one HuProt™ microarray from a plastic slide holder with fine-nosed tweezers, holding on to the side with the barcode.

   NOTE: The active surface of the HuProt™ microarray is also the barcoded side. The microarrays should be handled only at the barcoded end. Wear gloves and use tweezers to handle the microarrays.

4. Immediately, submerge the microarray in one well of the 4-well plate with the CDIArrayBlock™ buffer, with the active/barcoded side facing up.

5. When all four microarrays have been added to the 4-well plate, gently move the dish in a circular fashion until the blocking solution covers the entire surface of each array.

6. Incubate the arrays at room temperature for 1.0 hr. with gentle shaking (20-40 rpm).

5.6 Add the Samples to the HuProt™ Microarrays

1. **A. For antibodies, biotinylated proteins or small molecules:** Add 3 µg of each antibody or protein sample, or 30 µL of 1 mM small molecule sample to each well of the rectangular 4-well plates containing the HuProt™ arrays that are covered by CDIArrayBlock™ buffer.

   Add the samples onto the barcoded area of the blocked HuProt™ microarray (barcode facing up). Do not let the pipette tip touch the microarray surface.

   B. For serum/plasma samples:

   Carefully decant the liquid from the 4-well plate. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

   Then carefully pipette 3.0 mL of the prepared sample, diluted in Seromics™ Sample Buffer, from the tube onto the barcoded area of the blocked HuProt™ microarray (barcode facing up). Do not let the pipette tip touch the microarray surface.

2. Replace the plastic cover on the 4-well plate when all the test samples have been added.

3. Place the 4-well plates on an orbital shaker, and incubate at room temperature for 1 hour with gentle constant shaking (20-40 rpm). The covered 4-well plates may be stacked for this step.

4. If fluorescently labelled samples are being assayed, be sure to cover the 4-well plates with aluminum foil to minimize light exposure.

5.7 First Washing Stage

1. Following incubation, remove the cover from one of the rectangular 4-well plates.

2. Carefully decant the liquid from the 4-well plate into a designated waste container. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

3. RINSING: Slowly add 4.0 ml of 1X TBS-T solution to each well of the 4-well dish. Make sure to add the solution to the base of the array (over the barcoded area). Quickly rinse the microarray for 5 seconds. Decant the liquid from the 4-well plate into the designated liquid waste container. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

4. WASHING: Slowly refill each well with 4.0mL of 1X TBS-T solution. Make sure to add the solution at the base of the array (over the barcoded area). Place the 4-well plates on an orbital shaker and incubate at room temperature for 10 min with constant shaking (20-40 rpm). Decant the liquid from the 4-well plate into the designated liquid waste container. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

   NOTE: Always make sure that the wells are not left empty, or the microarrays will dry out. Repeat the steps for each rectangular 4-well dish.

5. Repeat for a total of three 10 min washes in 1X TBS-T.

5.8 Add Secondary Antibody (or Streptavidin)

IMPORTANT: For the following steps, cover the 4-well plates containing the HuProt™ microarrays tightly with aluminum foil to minimize light exposure.

IMPORTANT: Prepare the secondary antibody or streptavidin solution (3.5 mL per microarray) immediately before use.

1. Dilute the secondary antibody (for serum or antibody samples) or streptavidin (for biotinylated samples) in the Detection Buffer per manufacturer's directions for Western Blot use. Wrap the containers in aluminum foil.

2. Decant the liquid (TBS-T) covering the washed HuProt™ microarrays from the 4-well plate into a designated liquid waste container. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

3. Add 3.0 mL of the prepared secondary antibody solution to each HuProt™ microarray. Replace the cover on the 4-well plate after all the wells are filled.

4. Stack the rectangular 4-well plates and cover with foil to block light, or cover the individual 4-well plates with aluminum foil.

5. Place the covered 4-well plates on an orbital shaker. Shake gently (20-30 rpm) for 1.0 hr. at room temperature.

5.9 Second Washing Stage

1. Carefully decant the buffer containing the secondary antibody from the 4-well plate into a designated liquid waste container. The HuProt™ microarrays will stay attached to the bottom of the 4-well plate.

2. TBS-T RINSE: Slowly add 4.0ml 1X TBS-T over the barcoded end of the microarray, and briefly rinse the microarray for 5.0 seconds. Carefully decant the rinse buffer.

3. TBS-T WASHES: Slowly add 4.0 ml of 1X TBS-T over the barcoded end of the microarray and incubate for 10 min at room temperature with gentle shaking on an orbital shaker (20-30 rpm).

4. Carefully decant the wash buffer from the 4-well plate into a designated liquid waste container.

5. Repeat for a total of three 10 min washes in 1X TBS-T.

6. ddH2O RINSES: Carefully add 4.0 mL of ddH2O to the barcoded end of each microarray. Rinse for 5 seconds. Then carefully decant the ddH2O into a designated liquid waste container.

7. Repeat for a total of three rinses in ddH2O.

8. Leave the microarray in ddH2O after the third rinse and immediately proceed to the drying step.

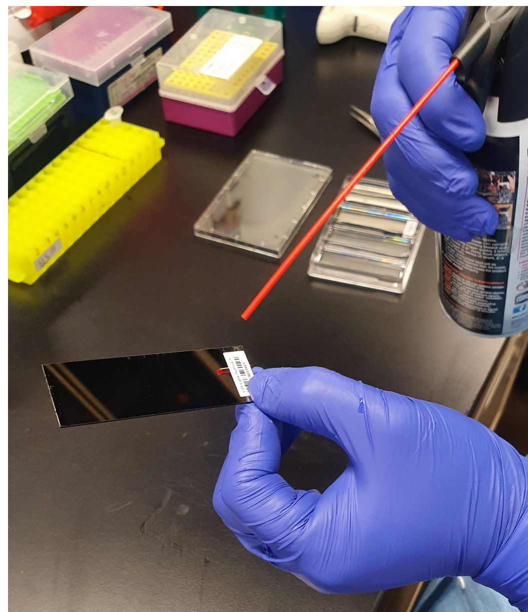
> **IMPORTANT:** Go to the drying stage immediately.

5.10 Microarray Drying Stage

1. Label a light-proof microscope slide box to store the microarrays.

2. Carefully use tweezers to remove one HuProt™ microarray (submerged in ddH2O) from the 4-well plate, by holding on to the barcoded area. Do not remove more than one microarray at a time.

3. Gently tap a corner of the microarray on a clean room wipe to wick away excess water.



4. Gently blow the microarray dry from the barcoded area, using a constant angled flow of compressed air.

5. Gently tap the edges and back of the microarray on a clean room wipe to wick away excess water.

6. Place each dried microarray inside the light-proof microscope slide box.

7. Store the microarrays in the order in which they were processed.

8. Once all the HuProt™ microarrays are dried and stored, immediately proceed to the scanning step.

9. Alternatively, store the light-proof slide box at 4°C overnight, and scan the microarrays the next day.

5.11 Microarray Scanning

1. The HuProt™ microarray should be scanned immediately after drying (highly preferable), or stored at 4°C in a lightproof microscope box overnight.

2. If the microarrays are stored overnight at 4°C, they must be scanned the next day. Place the covered lightproof microscope box containing the microarrays on a bench at room temperature for 30 minutes, to bring the arrays to room temperature before scanning.

6 RNA Binding Assays

IMPORTANT: Do not use the CDIArrayBlock™ or Seromics™ Sample Buffer for RNA binding assays.

The RNA samples should be fluorescently labeled.

6.1 Reagents for RNA Binding

Bovine Serum Albumin (IgG-Free, Protease-Free) (e.g. Jackson ImmunoResearch Laboratories 001-000-173)
10X TBS (Tris buffered saline)
Tween® 20
ssDNA (herring sperm)
MgCl2
SuperBlockT20 (PBS) Blocking Buffer (Thermo Scientific Cat. No. 37516)
DEPC-treated water
PBS

6.2 Recipes for RNA Binding

•**RNA Binding Assay Blocking Buffer:** 10 μg/mL ssDNA (herring sperm), 2 mM MgCl2, 5 mg/mL BSA in SuperBlock T20 (PBS) Blocking Buffer

•**RNA Binding Buffer:** 2 mM MgCl2; 2 mg/mL BSA (filtered with a 0.45 μm filtration system), 10 μg/mL ssDNA (herring sperm) in 1X PBS

•**Washing Buffer:** 1X TBS pH 7.4, 0.1% Tween™ 20. Commercially available 10X TBS may also be purchased. Store the buffer at room temperature.

6.3 HuProt™ Microarray Storage and Preparations

NOTE: Purified proteins are printed on the side of the HuProt™ microarray with the barcode to create the “active surface.” The barcode marks the end of the microarray.

1. Prepare the blocking and binding buffers and store on ice or at 4℃. Prepare the washing buffer.
2. Set aside the exact number of rectangular 4-well plates needed and label them with a permanent marker.
3. Store HuProt™ microarrays in closed plastic slide holders at -80℃ (each holder holds up to 5 HuProt™ arrays).
4. After removing plastic slide holders containing microarrays from -80℃ storage, place the plastic slide holders flat on a layer of dry ice, until the blocking stage.

IMPORTANT: Keep the HuProt™ microarrays ultra cold/ultra dry before blocking. Do not let condensation form on the microarray surface.

6.4 Block the HuProt™ Microarrays

1. Use a serological pipette to add 3.0 mL of RNA Binding Assay Blocking Buffer to each well in the rectangular 4-well plates. Each well will hold one HuProt™ microarray for the assays and subsequent steps.

   NOTE: Some users leave an unused, empty well in between each microarray to minimize cross contamination.
2. After the wells are filled with RNA Binding Assay Blocking Buffer, remove the plastic slide holders containing the HuProt™ arrays from the -80℃ freezer, and place the holders flat on a layer of dry ice. The barcodes on the microarrays should be facing up.
3. Carefully remove one HuProt™ microarray from a plastic slide holder with fine-nosed tweezers, holding on to the side with the barcode.

   NOTE: The active surface of the HuProt™ microarray is also the barcoded side. The microarrays should be handled only at the barcoded end. Wear gloves and use tweezers to handle the microarrays.
4. Immediately, submerge the microarray in one well of the 4-well plate with the blocking buffer, with the active/barcoded side facing up.
5. When all four microarrays have been added to the 4-well plate, gently move the dish in a circular fashion until the blocking solution covers the entire surface of each array.
6. Incubate the arrays at room temperature for 1 hr. with gentle shaking (20-40 rpm).

6.5 RNA Sample Preparation

1. Dilute the fluorescently labeled RNA sample to a concentration of 250 nM in 3.0 ml of RNA binding buffer (store the RNA binding buffer on ice or at 4℃ until ready to use; use aluminum foil to minimize light exposure once the RNA sample has been added). For all subsequent assay steps conducted in 4-well plates, cover the plates with aluminum foil to minimize light exposure.

6.6 Add the RNA Samples to the HuProt™ Microarrays

1. Carefully decant the liquid from the 4-well plate. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

2. Carefully pipette 3.0 mL of the prepared RNA sample (diluted in RNA binding buffer) onto the barcoded area of the blocked HuProt™ microarray (barcode facing up). Do not let the pipette tip touch the microarray surface.

3. Replace the plastic cover on the 4-well plate when all the test samples have been added. Cover the 4-well plates with aluminum foil to minimize exposure to light.

4. Place the 4-well plates on an orbital shaker, and incubate at room temperature for 1 hour with gentle constant shaking (20-40 rpm). The 4-well plates may be stacked and then covered with foil for this step.

6.7 Washing Stage

1. Following incubation, remove the cover from one of the rectangular 4-well plates.

2. Carefully decant the liquid from the 4-well plate into a designated waste container. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

3. RINSING: Slowly add 4.0 mL of 1X TBS-T solution to each well of the 4-well dish. Make sure to add the solution to the base of the array (over the barcoded area). Quickly rinse the microarray for 5 seconds. Decant the liquid from the 4-well plate into a designated waste container. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

4. WASHING: Slowly refill each well with 4.0 mL of 1X TBS-T solution. Make sure to add the solution at the base of the array (over the barcoded area). Cover the 4-well plates with foil and then place on an orbital shaker and incubate at room temperature for 10 min with constant shaking (20-40 rpm). Decant the liquid from the 4-well plate into a designated waste container. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

   NOTE: Always make sure that the wells are not left empty, or the microarrays will dry out. Repeat the steps for each rectangular 4-well dish. Be sure to cover the 4-well plates with aluminum foil at all times to minimize light exposure.

5. Repeat for a total of three 10 min washes in 1X TBS-T.

6. FINAL RINSES: Carefully add 4.0 mL of DEPC-treated water to the barcoded end of each microarray. Rinse for 5 seconds. Then carefully decant the water into a designated liquid waste container.

7. Repeat for a total of three rinses in DEPC-treated water.

8. Leave the microarray in DEPC-treated water after the third rinse and immediately proceed to the drying step.

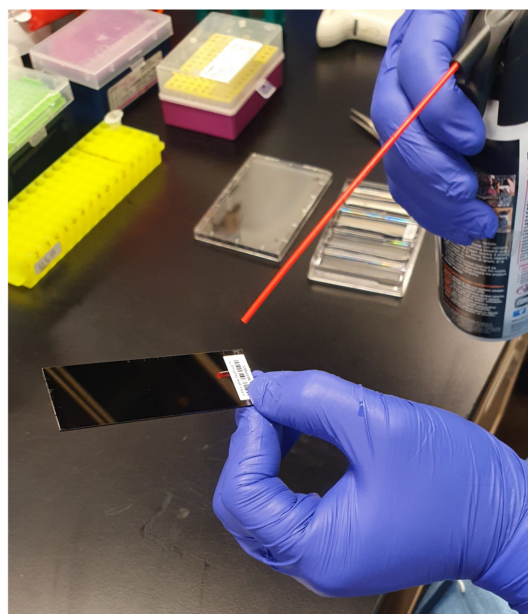
IMPORTANT: Go to the drying stage immediately.

6.8 Microarray Drying Stage

1. Label a light-proof microscope slide box to store the microarrays.

2. Carefully use tweezers to remove one HuProt™ microarray (submerged in DEPC-treated water) from the 4-well plate, by holding on to the barcoded area. Do not remove more than one microarray at a time.

3. Gently tap a corner of the microarray on a clean room wipe to wick away excess water.



4. Gently blow the microarray dry from the barcoded area, using a constant angled flow of compressed air.

5. Gently tap the edges and back of the microarray on a clean room wipe to wick away excess water.

6. Place each dried microarray inside the light-proof microscope slide box.
7. Store the microarrays in the order in which they were processed.
8. Once all the HuProt™ microarrays are dried and stored, immediately proceed to the scanning step.
9. Alternatively, store the light-proof slide box at 4℃ overnight, and scan the microarrays the next day.

6.9 Microarray Scanning

1. The HuProt™ microarray should be scanned immediately after drying (highly preferable), or stored at 4℃ in a lightproof microscope box overnight.
2. If the microarrays are stored overnight, they must be scanned the next day. Rest the lightproof microscope box containing the microarrays on a bench at room temperature for 30 min to bring the arrays to room temperature before scanning.

6.10 Appendix for RNA Binding Assay

Two tubes of buffer, CDIArrayBlock™ and Seromics™ Sample Buffer, are included with each HuProt v4.0 microarray purchased. Do not use these two buffers for RNA binding assays on HuProt™ microarrays. Please refer to the relevant protocols and recipes shown at the beginning of this protocol (Section 6).

7 DNA Binding Assays

IMPORTANT: Do not use CDIArrayBlock™ or Seromics™ Sample Buffer for DNA binding assays.

The DNA samples should be fluorescently labeled.

7.1 Reagents for DNA Binding

ddH2O
HEPES
Potassium glutamate
DTT
Glycerol
Poly (dA-dT)

7.2 Recipes for DNA Binding

DNA Hybridization Buffer: 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. Store on ice or at 4℃.

Washing Buffer: Same as above, but without poly (dA-dT). Store on ice or at 4℃.

7.3 HuProt™ Microarray Storage and Preparations

NOTE: Purified proteins are printed on the side of the HuProt™ microarray with the barcode to create the "active surface." The barcode marks the end of the microarray.

1. Prepare the DNA Hybridization Buffer and store on ice. Prepare the washing buffer.
2. Set aside the exact number of rectangular 4-well plates needed and label them with a permanent marker.
3. Store HuProt™ microarrays in closed plastic slide holders at -80℃ (each holder holds up to 5 HuProt™ arrays).
4. After removing plastic slide holders containing microarrays from -80℃ storage, place the plastic slide holders flat on a layer of dry ice, until the blocking stage.

IMPORTANT: Keep the HuProt™ microarrays ultra cold/ultra dry before blocking. Do not let condensation form on the microarray surface.

7.4 Block the HuProt™ Microarrays

1. Use a serological pipette to add 3.0 ml of DNA Hybridization Buffer (pre-chilled and stored at 4℃) to each well in the rectangular 4-well plates. Each well will hold one HuProt™ microarray for the assays and subsequent steps.

   NOTE: Some users leave an unused, empty well in between each microarray to minimize cross contamination.

2. After all the wells are filled with DNA Hybridization Buffer, remove the plastic slide holders containing the HuProt™ arrays from the -80℃ freezer, and place the holders flat on a layer of dry ice. The barcodes on the microarrays should be facing up.

3. Carefully remove one HuProt™ microarray from a plastic slide holder with fine-nosed tweezers, holding on to the side with the barcode.

   NOTE: The active surface of the HuProt™ microarray is also the barcoded side. The microarrays should be handled only at the barcoded end. Wear gloves and use tweezers to handle the microarrays.

4. Immediately, submerge the microarray in one well of the 4-well plate with the DNA Hybridization Buffer, with the active/barcoded side facing up.

5. When all four microarrays have been added to the 4-well plate, gently move the dish in a circular fashion until the blocking solution covers the entire surface of each array.

6. Incubate the arrays at 4℃ for 3 hours with gentle shaking (20-40 rpm).

7.5 DNA Sample Preparation

1. Dilute the fluorescently labeled DNA sample to a concentration of 40 nM in in 3.0 ml of DNA hybridization buffer containing poly (dA-dT) and store on ice until use. (use aluminum foil to minimize light exposure once the DNA sample has been added to the buffer). For subsequent assay steps conducted in 4-well plates, cover the plates with aluminum foil to minimize light exposure.

7.6 Add the Labeled DNA Samples to the HuProt™ Microarrays

1. Carefully decant the liquid from the 4-well plate. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

2. Carefully pipette 3.0 mL of the prepared DNA sample (diluted in DNA binding buffer) onto the barcoded area of the blocked HuProt™ microarray (barcode facing up). Do not let the pipette tip touch the microarray surface.

3. Replace the plastic cover on the 4-well plate when all the test samples have been added. Cover the 4-well plates with aluminum foil to minimize exposure to light.

4. Place the 4-well plates on an orbital shaker, and incubate at 4℃ overnight with gentle constant shaking (20-40 rpm). The covered 4-well plates may be stacked and then covered with foil for this step.

7.7 Washing Stage

1. One wash cycle is sufficient for the DNA Binding Assay

2. Following incubation, remove the cover from one of the rectangular 4-well plates.

3. Carefully decant the liquid from the 4-well plate into a designated waste container. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

4. WASHING: Add 4.0 mL of pre-chilled wash buffer (chilled at 4℃) to each well of the 4-well dish. Make sure to add the solution to the base of the array (over the barcoded area). Incubate with gentle shaking for 1-3 min at 4℃. Decant the liquid from the 4-well plate into the designated waste container. The HuProt™ microarray will stay attached to the bottom of the 4-well plate.

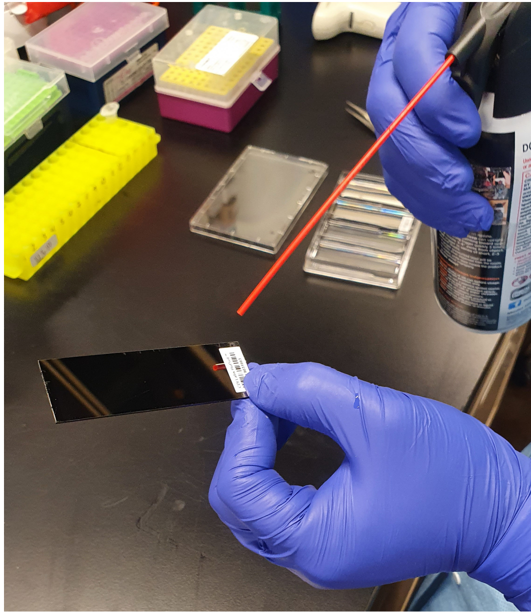
IMPORTANT: Check that no wells are left empty, or the microarrays will dry out. Repeat the steps for each rectangular 4-well dish. Cover the 4-well plates with aluminum foil at all times to minimize light exposure.

IMPORTANT: Go to the drying stage immediately.

7.8 Microarray Drying Stage

1. Label a light-proof microscope slide box to store the microarrays.

2. Carefully use tweezers to remove one HuProt™ microarray (submerged in wash buffer) from the 4-well plate, by holding on to the barcoded area. Do not remove more than one microarray at a time.



3. Gently tap a corner of the microarray on a clean room wipe to wick away excess water.
4. Gently blow the microarray dry from the barcoded area, using a constant angled flow of compressed air.
5. Gently tap the edges and back of the microarray on a clean room wipe to wick away excess water.
6. Place each dried microarray inside the light-proof microscope slide box.
7. Store the microarrays in the order in which they were processed.
8. Once all the HuProt™ microarrays are dried and stored, immediately proceed to the scanning step.
9. Alternatively, store the light-proof slide box at 4 ℃ overnight, and scan the microarrays the next day.

7.9 Microarray Scanning

1. The HuProt™ microarrays should be scanned immediately after drying (highly preferable), or stored at 4 ℃ in a lightproof microscope box overnight.
2. If the microarrays are stored overnight, they must be scanned the next day. Rest the lightproof microscope box containing the microarrays on a bench at room temperature for 30 minutes, to bring the arrays to room temperature before scanning.

7.10 Appendix for DNA Binding Assay

Two tubes of buffer, CDIArrayBlock™ and Seromics™ Sample Buffer, are included with each HuProt™ microarray. Do not use these buffers for DNA binding assays on HuProt™ microarrays. Please refer to the relevant protocols and recipes shown at the beginning of this protocol (Section 7).

8 HuProt™ References

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