PATH® Protein Microarray Kit

User Manual

105502 – Rev.001

| PATH® Protein Microarray Reagent Kit |

User Manual

Catalog Ref.:105502

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1. Introduction

Nitrocellulose is the leading immobilization surface for protein microarray applications. PATH® ultra-thin nitrocellulose substrates provide numerous advantages over functionalized glass or hydrogel slides such as low fluorescence background, higher binding capacity, stability of native protein conformation and biological activity, ease of use and long-term storage. These benefits translate into the best signal to noise providing optimal sensitivity along with accurate and reproducible results.

The PATH® Protein Microarray Kit is an optimized system enabling the user to obtain the full technical potential of the ultra-thin nitrocellulose substrate. The kit includes the all key reagents required for maximal use of PATH® ultra-thin nitrocellulose film slides and is suitable for a variety of applications such as antibody sandwich or direct capture assays as well as reverse phase protein assays (see Figure 1).

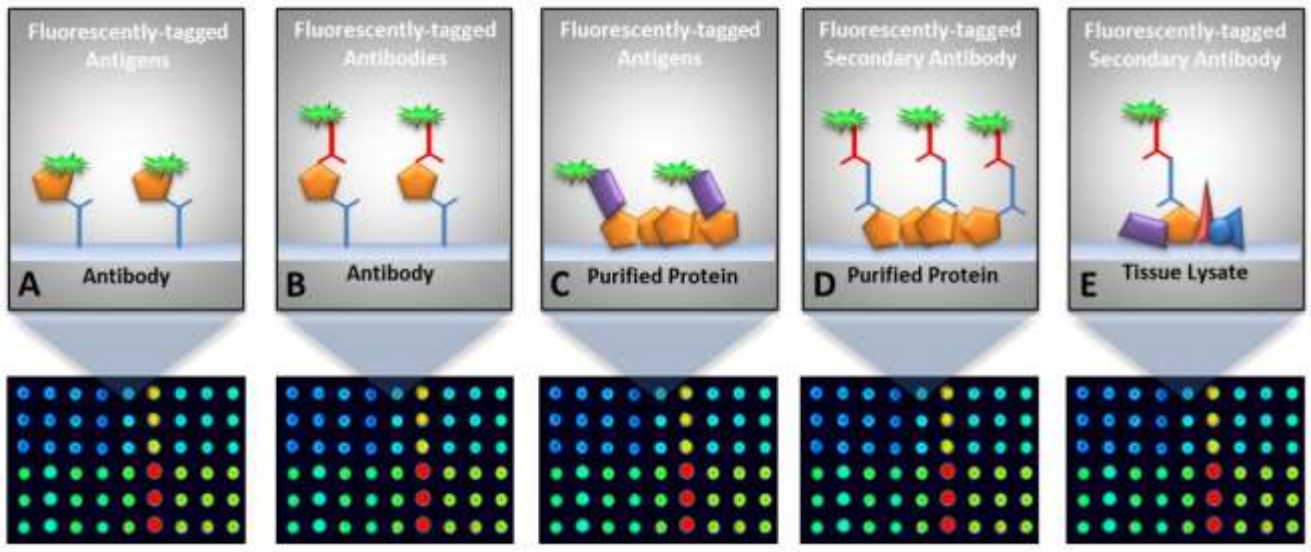

Figure 1. Commonly used protein microarrays configurations. (A) Antibody arrays can be used to capture specific antigens which are directly labeled or (B) used in sandwich ELISA-like assays. (C) Purified or recombinant proteins can be arrayed to study protein-protein interaction or (D) to probe serum samples for antibodies. (E) Reverse-Phase Protein Arrays (RPPA) are used to profile dozens or hundreds of arrayed samples (e.g. cell or tissue lysates) for the presence of selected antigens.

The components of the kit are specifically engineered to function with the PATH® slide as a system to provide:

- Low, non-specific binding background
- Broad and quantitative dynamic detection ranges
- Optimal signal to noise
- Maximum immobilized protein stability

- Reproducible spot morphology
- Consistent inter- and intra-assay results
- Flexible slide layout for a variety of applications
- Easy-to-use and validated methods
- Easy to handle and high throughput slide/plate configurations

2. Intended Use

This PATH® Protein Microarray Kit is designed to provide users with reagents and methods necessary for the preparation and use of protein microarrays derived from various sources including purified antibodies and antigens. The reagents in this system supplement the use of PATH® ultra-thin nitrocellulose film slides and are provided in quantities for processing up to 20 film slides with our ProPlate® Microarray Incubation Chambers.

For Research Use Only

3. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit please refer to the Safety Data Sheet (SDS).
- Kit cannot be used beyond the expiration date on the label.

4. Storage and Stability

Upon receipt store components at the indicated conditions below. Do not use the kit beyond the expiration date. Store tightly sealed in original container and protected from direct sunlight in a dry, cool and well-ventilated area, between the following temperatures: 20 to 25°C. See instructions for storage and stability for the prepared reagents in Section 8, Reagent Preparation.

5. Kit Components

- The following items are provided with the kit:

Catalogue #	Component	Qty	Storage
105407	**4X PATH® Print Buffer**	1 x 50 mL	Store at ambient temperature until date on kit
105207	**2X PATH® Rinse Buffer**	2 x 250 mL	
105111	**2X PATH® Blocking Buffer**	1 x 250 mL	
105208	**4X PATH® Sample Diluent**	1 x 150 mL	
105209	**4X PATH® Wash Buffer**	1 x 250 mL	

6. Required Materials Not Supplied

- ProPlate® Slide Chamber System – variety of configurations
- Scanner capable of measuring fluorescence based on the selected detection fluorophore.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Protease free distilled or deionized ultrapure water.
- Glass or metal slide staining rack and chamber
- Staining dish and staining rack
- Squirt bottle

7. Technical Application Tips

- Do not mix or substitute components from other kits.
- Replicate wells are recommended for all samples.
- Cover slides with lids or seal Proplate® with seal stripes while incubating to prevent evaporation.
- Do not allow the slide wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove all liquids when washing to prevent cross contamination.

- When aspirating liquid from the well, do not touch the array. Place pipet tip in the corner of the well when aspirating.
- Equilibrate all materials to ambient room temperature prior to use.
- Pipetting less than 1 µL is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure all reagents, materials and devices are ready at appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples containing precipitates, fibrin strands, bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.
- Fluorophores are easily contaminated. Handle carefully and protect from light.

ProPlates® are designed to be used with the following volumes for the respective operations:

ProPlate® Format	**Grace Bio-Labs Catalogue #**	**Incubation Volume (µL)**	**Wash Volume (µL)**
1 Well (1 x 1)	248861, 246881	1,000 - 1,500	1,500 - 2,000
2 Wells (1 x 2)	248862, 246882	800 - 1,000	1,000 - 1,500
4 Wells (1 x 4)	248864, 246884	400 - 500	500 - 750
8 Wells (1 x 8)	248868, 246888	200 - 250	250 - 400
16 Wells (2 x 8)	244862, 246880	75 - 150	150 - 300
24 Wells (3 x 8)	472756, 246824	50 - 100	100 - 200

***** Several additional catalogue and custom configurations available.

8. Reagent Preparation

- Precipitation may occur during storage, particularly for **4X PATH® Print Buffer**. If crystals are observed, gently warm the bottle by briefly placing into a 30 to 40 ºC water bath or a warming plate and stir at 300 rpm for approximately 10-15 minutes (Stirbar is pre-packed in the **4X PATH® Print Buffer** bottle). All crystals should go back to solution.
- Equilibrate all reagents to room temperature prior to use.

The following quantities are sufficient to process approximately 10 slides:

Stock Reagent	Stock Reagent Concentration	Volume of Stock Reagent (mL)	Volume of ultrapure water (mL)	Final Volume of 1X reagent (mL)
4X PATH® Print Buffer	4X	3	9	12
2X PATH® Blocking Buffer	2X	125	125	250
4X PATH® Wash Buffer	4X	125	375	500
4X PATH® Sample Diluent	4X	75	225	300
2X PATH® Rinse Buffer	2X	250	250	500

Do not store diluted reagent for future use.

9. Microarray Construction and Preparation

9.1 Protein Printing Sample Preparation

- Arraying Protein Concentrations - The optimal concentration for proteins arrayed onto PATH® ultra-thin nitrocellulose surface is typically slightly less than those required for porous nitrocellulose or activated glass surfaces. Recommended starting concentration range is 500 µg/mL to 1 µg/mL. Optimal concentrations can be affected by the individual protein, spotting volume and spotting instrument method. Optimal condition of each protein arrayed must be derived by users for specific applications.
- Protein Arraying Formulations - PATH® Print Buffer is specifically formulated to enhance protein binding, minimize protein aggregation, promote proper protein folding, provide consistent spot morphology, reduce inconsistent backgrounds and support the long-term stability of arrayed proteins. For optimal performance protein samples should be suspended at a final concentration of **1X PATH® Print Buffer**.

  Two methods for preparing protein samples for printing protein samples in **1X PATH® Print Buffer** are included here;

  a) Reconstitution of lyophilized protein

  - or -

  b) Dilution of protein already in solution

Reconstitution of Lyophilized Protein using 1X PATH® Print Buffer:

9.1a.1 Prepare **1X PATH® Print Buffer** as indicated in section 8

9.1a.2 Resuspend lyophilized protein to desired stock storage concentration by adding the appropriate volume of **1X PATH® Print Buffer**. Allow at least 15 minutes for the protein to fully reconstitute.

9.1a.3 Mix gently and thoroughly using the pipet tip or inversion. Stock protein may be stored long term when suspended in **1X PATH® Print Buffer** at -20°C. Aliquot and avoid freeze/thaw cycles.

9.1a.4 For arraying, adjust the concentration of the stock protein which was reconstituted using **1X PATH® Print Buffer** to the final arraying concentration by diluting with **1X PATH® Print Buffer**.

9.1a.5 Mix gently and thoroughly using the pipet tip. Do not store the diluted final concentration protein for future use.

Dilution of Suspended Protein using 4X PATH® Print Buffer:

9.1b.1 Adjust the concentration of previously suspended stock protein (not originally suspended in **1X PATH® Print Buffer**) to the final arraying concentration by diluting with ultrapure water and **4X PATH® Print Buffer** such that the final concentration is **1X PATH® Print Buffer**.

9.1b.2 Mix gently and thoroughly using the pipet tip. Do not store the diluted final concentration protein for future use.

Determine the volume of required reagents for suspension of proteins in 4X PATH® Print Buffer as follows:

Volume Stock Protein = (Final Arraying Protein Concentration/Stock Protein Concentration) x Total Required Arraying Volume

Volume 4X Print Buffer = Total Required Volume / 4

Volume Ultrapure Water = Total Required Volume – (Volume 4X Print Buffer + Volume Stock Protein)

Example:

Stock Protein Concentration = 1,000 µg /mL
Final Arraying Protein Concentration = 100 µg/mL
Total Arraying Volume Required = 100 µL
Volume Stock Protein = 10 µL
Volume **4X PATH® Print Buffer** = 25 µL
Volume Ultrapure Water = 65 µL

9.2 Microarray Printing

The PATH® ultra-thin nitrocellulose surface requires specific temperature and humidity conditions for optimal performance. Due to the wide variety of arraying instrumentation and protein samples, users must derive optimal conditions for their individual systems.

General specifications for optimally arraying protein onto PATH® slides along with good standard practices for arraying processes follows:

- Slide Pre-Treatment - PATH® slides require no pre-treatment or activation prior to arraying with protein. Place the slides into the arraying instrument 1 hour prior to arraying to allow the slides to equilibrate to the recommended arraying environmental conditions.
- Environmental Conditions – Ambient temperature, 50 – 60% humidity. Protein samples should be held chilled (on ice, refrigerated or on a cooling block at +4°C) and covered to prevent degradation or evaporation.
- Printing Buffer - Proteins arrayed on PATH® slides should be suspended in a final concentration of **1X PATH® Print Buffer** (See section 9.1).
- Arraying Concentrations - The optimal concentration for proteins arrayed onto PATH® ultra-thin nitrocellulose surface is typically slightly less than those required for porous nitrocellulose or activated glass surfaces. Recommended starting concentration range is 500 µg/mL to 1 µg/mL. Optimal concentrations can be affected by the individual protein, spotting volume and spotting instrument method. Optimal conditions must be derived by users for specific arraying methods and assay applications for each protein arrayed.
- Spot Size - Diameter of spot sizes will be dependent on arraying instrumentation, protein formulations, temperature and humidity. Typical observed spot sizes for PATH® ultra-thin nitrocellulose surfaces are:
    - Non-contact arraying - 150 µm to 200 µm using a 550 pL droplet volume
    - Contact arraying - Dependent on spotting tip configuration.
- Post-Arraying Slide Processing - Place slides at arraying environmental conditions for at least one hour after arraying to maximize the immobilization. Slides may be used immediately after arraying but may not perform optimally. Unused slides should be kept in a sealed and desiccated container at 4°C for future use.

  A curing period is recommended prior to use of arrayed slides. Slides should be placed into a sealed and desiccated container at room temperature or at 4°C for 24 hours minimally. Curing at room temperature for 3-5 days or at 4°C for 1 week prior to use will provide benefits for some immobilized proteins.
- Arrayed Slide Storage and Stability - Store protein arrayed slides in a sealed, desiccated container at +4°C or -20°C. Stability is protein dependent. Slides can be blocked prior to storage for added stability (see Section 9.3)

- Pre-Spin – Prepared proteins for printing should be centrifuged briefly at 2,000 rpm to remove any precipitates or particulates that would interfere with the printing instrumentation.

9.3 Microarray Blocking

PATH® Blocking Buffer is a non-proteinaceous solution specifically formulated to inhibit non-specific binding and prolong stability of arrayed proteins. Blocking procedures should be followed precisely to obtain microarrays with optimal signal to noise and minimal background artifacts when imaging.

- PATH slides are blocked most effectively when the first step of buffer application is performed rapidly and with a copious volume of liquid.
- Prior to blocking, slides should be removed from storage and equilibrated to ambient conditions for 1 hour in a desiccated container.

Method A – Blocking in ProPlate® Wells

- Recommended when processing a small number of slides for immediate use after blocking.

9.3a.1 Using a squirt bottle with a wide liquid stream, rigorously spray a printed slide using approximately 40-50 mL of **1X PATH® Rinse Buffer**. Spray the slide completely and thoroughly. Do not allow the slide to dry.

9.3a.2 Immediately spray the slide completely and thoroughly with another 40-50 mL of deionized water.

9.3a.3 Dry the slide using a gentle stream of compressed nitrogen or filtered air. Place the slide on end upon a laboratory KimWipe to completely dry.

9.3a.4 Affix the ProPlate® Chamber.

9.3a.5 Add **1X PATH® Blocking Buffer** to each well of the chamber. (ProPlate® well incubation volumes listed in Section 6).

9.3a.6 Incubate the **1X PATH® Blocking Buffer** for 15 minutes stationary.

9.3a.7 Remove the liquid with aspiration or flicking into a waste container.

9.3a.8 Gently tap the slide/ProPlate® assembly inverted onto a paper towel on the bench-top. **Do not allow wells to complete dry at any step in the washing process.**

9.3a.9 Wash the wells by adding **1X PATH® Wash Buffer** to the wells (ProPlate® well wash volumes listed in Section 7) and incubate for 5 minutes with agitation.

9.3a.10 Proceed to assay processing steps (Section 10.3)

Method B – Blocking in a Chamber

- Recommended when processing a large number of slides simultaneously or blocking slides for storage.

9.3b.1 Fill an appropriate staining dish with approximately 250 mL of **1X PATH® Rinse Buffer**.

9.3b.2 Place the slides into a staining dish rack and rapidly plunge the slide into the liquid. (Faster insertion of the slide into the liquid will yield superior results).

9.3b.3 Gently but thoroughly agitate rack with the slides in the liquid up and down 10 times.

9.3b.4 Transfer to another staining dish filled with approximately 250 mL of **1X PATH® Blocking Buffer**

9.3b.5 Incubate at room temperature for 15 minutes stationary.

9.3b.6 Remove the slides and rinse the slides briefly with deionized water.

9.3b.7 Dry under a gentle stream of compressed nitrogen gas or set on edge to air dry.

9.3b.8 Slides may be used immediately or stored desiccated for future use.

10. Assay Procedures

PATH® Slides can be utilized in numerous application methods. The general outline for most PATH® protein microarray experimental procedures are seen in Figure 2.

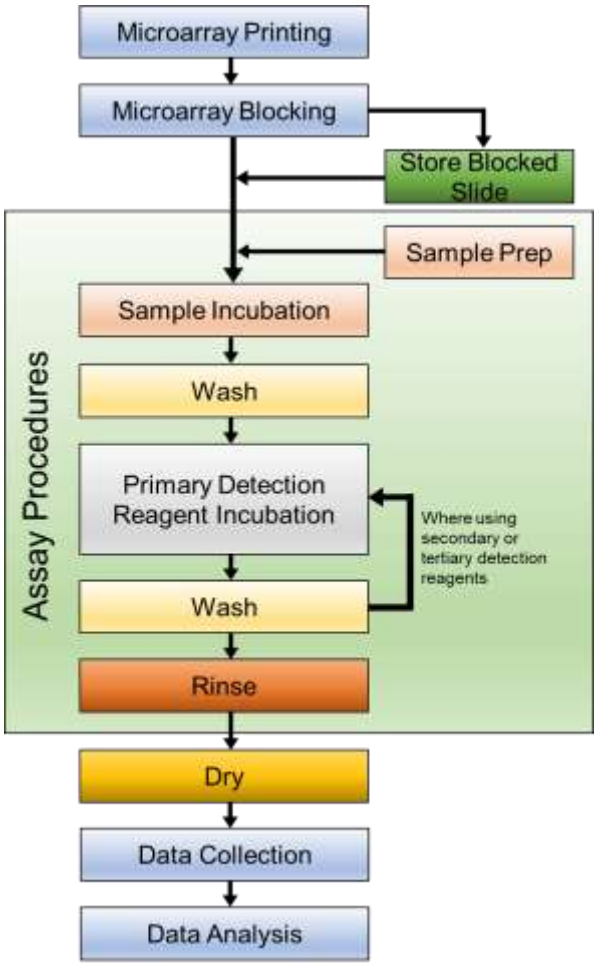

Figure 2. Outline for PATH® protein microarray experimental procedures.

10.1 Slide Preparation

- Equilibrate arrayed or pre-blocked slides from storage to ambient room temperature for 1 hour prior to starting assay. Slides should remain desiccated during this equilibration time to prevent surface condensation.

10.2 Sample Preparation

- Samples should be diluted in **1X PATH® Sample Diluent**.
- 1% Bovine Serum Albumin (w/v) can also be added into **1X PATH® Sample Diluent** if extensive nonspecific binding is observed.
- Optimal sample dilution must be determined for individual assays.
- Prepare sample dilutions immediately prior to use, do not hold diluted for greater than 1 hour or store for future use.

10.3 Assay Protocol

- **Do not allow slide surface to dry between any steps of the assay protocol.**
- Optimal incubation time for sample and detection reagents may vary for specific individual user's systems. The incubation times indicated for sample and detection reagents are observed general best conditions. User should optimize.

10.3.1 Add samples directly to ProPlate® chamber wells and incubate for 1 hour with gentle rotary agitation at 250 rpm (specific ProPlate® well incubation volumes listed in Section 7).

Note: Seal the ProPlate chamber with seal strip for longer incubations (>1 hour) to prevent evaporation.

10.3.2 Remove the liquid by aspiration or flicking into a waste container and gently tap the slide/ProPlate® assembly onto paper toweling on the benchtop. **Do not allow wells to complete dry at any step in the washing process.**

10.3.3 Immediately wash the wells three times:

- Add **1X PATH® Wash Buffer** to the wells (specific ProPlate® well wash volumes listed in Section 7)
- Incubate for 5 minutes with gentle agitation at 250 rpm.
- Remove the liquid by aspiration or flicking into a waste container.
- Gently tap the slide/ProPlate assembly onto paper toweling on the benchtop.

10.3.4 Add primary detection reagent and incubate for 1 hour with gentle rotary agitation at 250 rpm.

10.3.5 Remove the liquid by aspiration or flicking into a waste container and gently tap the slide/ProPlate® assembly onto paper toweling on the benchtop.

10.3.6 Wash wells three times as in 10.3.3.

Note: If using secondary or tertiary detection reagents, repeat steps 10.3.4 through 10.3.6 using the reagent as needed.

10.3.7 Rinse the wells 1 time with deionized water:

- Briefly rinse the wells by adding deionized water to the wells (specific ProPlate® well wash volumes listed in Section 7)
- Remove the liquid by aspiration or flicking into a waste container.

- Gently tap the slide/ProPlate® assembly onto paper toweling on the benchtop.

10.3.8 **Gently** remove the ProPlate® chamber while the slide is still wet.

10.3.9 Centrifuge to dry or dry the slide under a gentle stream of compressed nitrogen. If any salt crystals remain on the surface, **briefly** rinse using ultrapure water and dry. Slides are now ready to be imaged.

11. Appendices

11.1 Protease Inhibitors - For users requiring protease inhibitors for their protein solutions the recommended inhibitor cocktails are listed below. Working concentrations are 1 tablet per 10 ml Arraying Buffer volume.

Complete® Mini Protease Inhibitor Cocktail tablet (Roche Applied Sciences, Cat. # 11 836 153 001)

PhosSTOP® Phosphatase Inhibitor Cocktail (Roche Applied Sciences, Cat. # 04 906 837 001)