



**Reverse-Phase Protein Array (RPPA)
Assay System**

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Introduction

The Reverse-Phase Protein Array (RPPA) assay platform has become a powerful tool for protein expression profiling, biomarker discovery, and cancer diagnostics. Although primary efforts with this method have focused on cancer tissue profiling, this method has general utility for monitoring protein expression in experimental systems biology.



RPPA with colorimetric endpoint detection containing 1056 individual samples, each represented by five 2-fold serial dilutions. The array contains 6,336 spots configured as 48 spot grids (11 × 11 spots per grid) including 528 replicated controls. Image provided courtesy of the MD Anderson Cancer Center.

In conventional “Forward-Phase” protein array applications, individual microarray elements contain homogenous “bait” proteins (such as capture antibodies for specific biomarkers) used for detection of specific biomarkers in a sample of mixed proteins. In contrast, RPPAs are configured so that the microarray elements are comprised of a mixture of proteins from complex biological samples (in the form of cellular or whole tissue lysates) which are then probed for specific biomarkers with antigen-specific antibodies. In this format, RPPAs enable highly parallel biomarker profiling from cell/tissue lysates with quantitative detection of rare proteins or protein modifications (such as key phosphorylation events) from hundreds to thousands of distinct biological samples in a single assay.

The RPPA Assay System from Grace Bio-Labs was designed to provide users with an effective and easy-to-implement introduction to RPPA technology. The standardized RPPA reagents provided in this kit offer an effective system for generating high-quality data from RPPA assays. In addition, use of this kit can contribute to consistent results between assays, laboratories, and institutions.

Successful implementation of RPPA technology requires three key determinants: effective protein extraction and preservation, robust protein binding onto the microarray surface, and assay methods optimized for sensitive detection of protein components. With these factors in mind, this kit was designed to work in conjunction with ONCYTE® nitrocellulose film slides from Grace Bio-Labs, which are ideally suited for RPPA due to their unsurpassed protein binding capacity (40 µg protein per cm²). This characteristic has made ONCYTE® films the industry standard for RPPA applications.

The lysis reagents and methods provided in this kit include formulas previously cited in the literature and used for RPPAs using various sample types. Since its seminal application in tumor tissue profiling from laser capture micro-dissected

samples (1), numerous variations on the method have been employed for RPPA protein profiling from various tissues and cell types including excised tissues, biopsy samples, and cultured cells (2-4). Differences in lysis reagents and methods can result in varying degrees of protein extraction, a result of diverse protein chemistries, cellular localization, and tissue types. Although some work has been performed to assess lysis buffer performance (5), a thorough evaluation of lysis buffers with various sample types remains to be done.

Grace Bio-Labs has developed a lysis buffer (also used as a spotting buffer) which has been optimized to work with our ONCYTE® films for maximizing RPPA signal. In addition, this kit provides the reagents and recipes for a number of other lysis formulas which have been used extensively for RPPA from a number of tissue/cell types, thus allowing the researcher to discover the optimal buffer for their application.

RPPA assays are currently performed using various amplification and detection methods employing colorimetric, chemiluminescence, or fluorescence endpoints for detection. Regardless of the specific assay methods employed, effective blocking of porous nitrocellulose is of paramount importance for sensitive detection of specific proteins. Supplied in this kit are two blocking reagents optimized for RPPA with our ONCYTE® films to minimize non-specific protein binding and produce low background to maximize assay sensitivity.

The use of standardized substrates and reagents provided with this kit will facilitate high-quality data for RPPA assays and provide uniformity across the RPPA community. As RPPA methods progress further into clinical applications, consistent and sensitive results leading to reliable diagnosis will be of the utmost importance.

References:

1. Paweletz, CP. *et al.* (2001). Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. *Oncogene* , 20,1981-1989.
2. Nishizuka, S. *et al.* (2003). Proteomic profiling of the NCI-60 cancer cell lines using new high-density reverse-phase lysate microarrays. *Proc. Natl. Acad. Sci.* Nov 25;100(24):14229-34.
3. Tibes, R. *et al.* (2006). Reverse phase protein array: validation of a novel proteomic technology and utility for analysis of primary leukemia specimens and hematopoietic stem cells. *Mol. Cancer Ther.* 5:2512–21.
4. Sevecka, M. *et al.* (2011). Lysate microarrays enable high-throughput, quantitative investigations of cellular signaling. *Mol. Cell Proteomics* Apr;10(4):M110.005363.
5. Winters M. *et al.* (2007). Constitution and quantity of lysis buffer alters outcome or reverse phase protein microarrays. *Proteomics*, Nov;7(22):4066-8.

Intended Use and Storage

Intended Use

This Reverse-Phase Protein Array (RPPA) Assay System is designed to provide users with reagents necessary for the preparation and use of protein microarrays derived from cell and tissue lysates. The reagents in this system supplement the use of ONCYTE® porous nitrocellulose film slides and are provided in quantities for processing up to 20 film slides.

Sample Preparation and Microarray Preparation: This system provides components for preparation of common lysis buffers used in RPPA applications from various sample types including cells and tissues (including laser-dissected samples.) The lysis reagents in this kit should be prepared with protease and phosphatase inhibitors (not included) to provide the final lysis buffer for cell and tissue processing. Protocols in this manual will yield cell/tissue lysates with denatured, stabilized protein suitable for printing protein microarrays. Microarray printing may be performed directly after lysis or after storage of the lysed samples at -80°C.

RPPA Assay: Reagents are provided for the processing of printed RPPA nitrocellulose film slides, including denaturation of spotted protein, blocking of the nitrocellulose film, and recommended washing steps.

Note: For Research Use Only

Storage Conditions

The supplied kit components require variable storage conditions and have been segregated into two **Reagent Boxes**. Upon receipt, remove **Reagent Box 2** from the packaging and transfer to 4°C storage. Reagents in **Reagent Box 1** should be stored at room temperature, unless marked otherwise. Check all reagent bottles for recommended storage conditions. All reagents may be stored at recommended temperatures for up to 24 months, except as noted below. Some reagents may precipitate during shipping and/or after storage. Mix contents well before using. For buffers which may have precipitated, place vials into a 37° C water bath with occasional mixing until contents are fully re-dissolved (usually within 10 minutes).

Kit Contents

Store at Room Temperature

Store at 4°C

Reagent Box 1	Reagent Box 2
Lysis Buffer Reagent A (10 ml)	Lysis Buffer Reagent E, Part 2 (6 ml)*
Lysis Buffer Reagent B (10 ml)	Antigen Retrieval Buffer (15 ml)
Lysis Buffer Reagent C (10 ml)	Blocking Buffer 1 (100 ml)
Lysis Buffer Reagent D (10 ml)	Blocking Buffer 2 (100 ml)
Lysis Buffer Reagent E , Part 1 (Powdered Reagent)*	PBST (100 ml)**
Lysis Buffer Reagent F (10 ml)	TBST (100 ml)**

Notes:

*Upon mixing Parts 1 and 2 of this reagent, it should be aliquoted and stored at -20°C. Shelf-life for this reagent is 6 months once components are mixed.

**Due to their high concentrations, these buffers may be most likely to precipitate during shipping/storage. If necessary, follow general recommendations above to re-dissolve buffers prior to using. Once diluted to a 1x working concentration, buffer contents will not precipitate.

Materials and Reagents Not Provided in this Kit

Materials
ONCYTE® porous nitrocellulose film slides
ProPlate™ Microarray Incubation Chambers
0.2 µm syringe filter and syringe

Lysis Reagents
Protease and Phosphatase Inhibitors (see Appendix for recommendations)
Sterile, protease-free water (Sigma W4502, or equivalent)

Assay Reagents
Primary and Secondary and Labeled Antibodies
Amplification or Detection Reagents (Dako K1500, or equivalent)
Sterile, protease-free water (Sigma W4502, or equivalent)

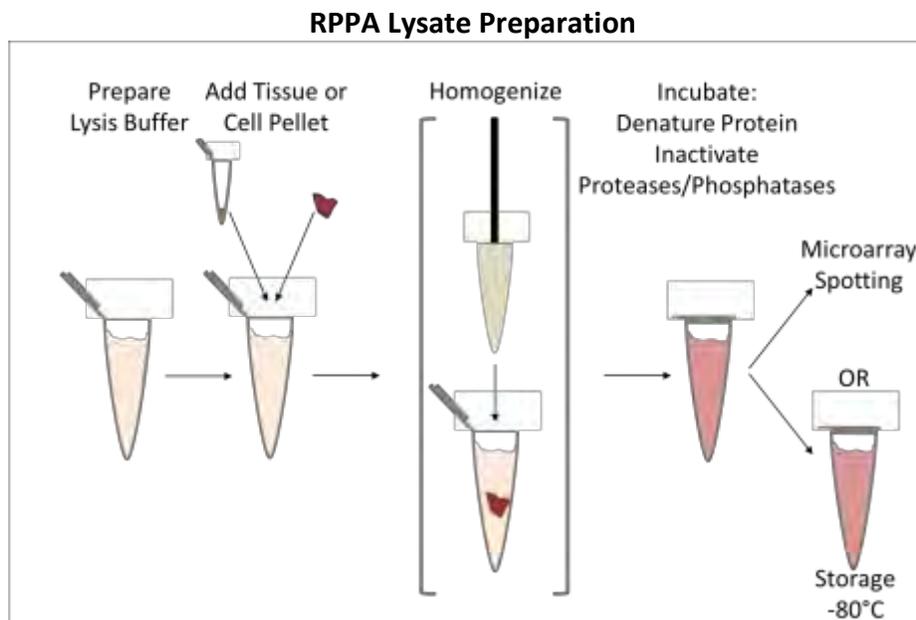
Protocols

Sample Preparation:

All lysis buffer recipes provided have been used for protein extraction for RPPA from various tissue types, cell culture, and laser-capture dissected samples (see Refs. 1 – 4). Extraction efficiency may vary with different lysis reagents and is dependent on the tissue being examined, the subcellular localization of biomarkers being detected, or both of these factors. For this reason, we have included reagents which can be used to create various recipes of commonly used RPPA lysis buffers to determine the most effective lysis/extraction conditions for the tissues and markers being studied. Unless working from *a priori* knowledge of an optimal protocol for your application, it is recommended to test the buffers supplied in this kit to determine the best conditions for your samples.

Lysis reagents should be supplemented with freshly added protease inhibitors prior to adding to your sample. If assessing phosphorylated proteins, supplementing with phosphatase inhibitors is also recommended. Recipes for stock solutions of the different protease and phosphatase inhibitors used in these formulations are listed in Appendix B. These should be prepared and aliquoted prior to beginning with lysis incubations. Additionally, recommended inhibitor cocktail reagents from Roche Life Sciences are also listed in Appendix B.

For effective lysis of samples we recommend using 1 ml lysis buffer per 40 mg of tissue or 3×10^6 cells. Complete lysis of some tissues may require mechanical disruption with use of a Dounce homogenizer or a bead-based homogenization system. Optimal conditions for lysis should be determined for your application.



The reagents provided are formulated to be used for the following lysis buffer options:

- I. Tris-HCl/Glycerol/SDS
- II. Tris-HCl/Glycerol/SDS/TPER
- III. HEPES/Triton X-100
- IV. Urea/CHAPS
- V. GBL Lysis Buffer

*See Appendix C for final formulation concentrations

Buffer Preparation and Lysis:

All volumes listed below are for the preparation per 1 ml of lysis buffer at their working lysis/extraction concentrations. Upon addition of protease and phosphatase inhibitors, it is highly recommended to perform lysis steps immediately as some inhibitors are inactivated within 30 min. of addition.

I. Tris-HCl/Glycerol/SDS Lysis Buffer:

1. Mix 0.125 ml each of Lysis Reagent A and B.
2. Add 0.250 ml protease-free water.
3. Add inhibitor stocks (prepare separately, not included in kit). Recommended:
 - a. 100 μ l 0.5M Sodium Fluoride
 - b. 5 μ l 1 M beta-glycerophosphate
 - c. 5 μ l 0.2 M Na_3VO_4
 - d. 5 μ l 0.2 M PMSF
 - e. 10 μ l 0.1 M DTT
 - f. 375 μ l water
 - g. Note: This brings volume up to 1 ml.
4. Add any additional inhibitors (see Appendix B).
5. Mix appropriate lysis buffer volume for cells/tissues to be lysed. (1ml/40mg sample or 3×10^6 cells)
6. Vortex for 15 seconds or perform mechanical tissue disruption.
7. Incubate for 15 minutes at room temperature.
8. Boil the cell lysate for 10 minutes at 100°C.
9. Centrifuge at 14,000 rpm for 5 min at 4°C.
10. Remove supernatant and discard pellet.
11. Proceed to microarray spotting or store lysate at -80°C until microarray spotting.

II. Tris-HCl/Glycerol/SDS/TPER Lysis Buffer:

1. Mix 0.250 ml each of Lysis Reagent A and protease-free water.

2. Add 25 μ l of β -mercaptoethanol.
3. Add 0.5 ml of Lysis Reagent C (TPER).
 - a. Add any additional inhibitors if necessary (optional, see Appendix B).
 - b. If additional protease and phosphatase inhibitors are to be used, add to the TPER volume prior to mixing.
 - c. Note: This brings volume up to 1 ml.
4. Mix appropriate lysis buffer volume for cells/tissues to be lysed. (1ml/40mg sample or 3×10^6 cells)
5. Vortex for 15 seconds or perform mechanical tissue disruption (if necessary).
6. Incubate for 15 minutes at room temperature.
7. Boil the cell lysate for 10 minutes at 100°C.
8. Centrifuge at 14,000 rpm for 5 min at 4°C.
9. Collect the supernatant and discard pellet.
10. Proceed to microarray spotting or store lysate at -80°C until microarray spotting.

III. HEPES/Triton X-100 Lysis Buffer:

1. To 0.5 ml Lysis Reagent D, add:
 - a. 200 μ l 0.5 M Sodium Fluoride
 - b. 100 μ l 0.1 M Sodium Pyrophosphate
 - c. 5 μ l 0.2 M Na_3VO_4
 - d. 195 μ l protease-free water
 - e. Note: This brings volume up to 1 ml.
2. Add any additional inhibitors if necessary (optional, see Appendix B).
3. Cool lysis buffer on ice before lysis.
4. Mix appropriate lysis buffer volume for cells/tissues to be lysed. (1ml/40mg sample or 3×10^6 cells)
5. Vortex for 15 seconds or perform mechanical tissue disruption (if necessary) on ice.
6. Incubate on ice for 20 min.
7. Centrifuge at 14,000 rpm for 10 min at 4°C.
8. Collect the supernatant and discard pellet.
9. Mix 3 parts lysate to 1 part Lysis Reagent A.
 - a. Add 1:10 volume β -mercaptoethanol to Lysis Reagent A prior to adding to lysate.
10. Boil the cell lysate for 5 minutes at 100°C.
11. Proceed to microarray spotting or store lysate at -80°C until microarray spotting.

IV. Urea/CHAPS Lysis Buffer:

Prepare Buffer:

1. Transfer 6 ml of Lysis Reagent E Part II to Lysis Reagent E Part I (urea).
2. Cap and mix gently by rocking for 30 minutes or until contents are fully dissolved.
 - a. This reaction is endothermic so contents will be cold to the touch.
 - b. Do not mix vigorously to avoid excessive detergent foaming.

- c. Do not heat the buffer as this will result in degradation of urea.
3. Add 0.4 g DTT and mix gently until dissolved.
4. Filter final solution with a 0.2 μm syringe filter into 1 ml aliquots and freeze at -20°C until use. (Store up to 6 months)

For lysis:

1. Thaw vial(s) of lysis buffer as needed.
2. Add any additional inhibitors if necessary (optional, see Appendix B).
3. Mix appropriate lysis buffer volume for cells/tissues to be lysed. (1ml/40mg sample or 3×10^6 cells)
4. Vortex for 15 seconds or perform mechanical tissue disruption (if necessary) on ice.
5. Incubate on ice for 20 min.
6. Centrifuge at 14,000 rpm for 10 min at 4°C .
7. Collect the supernatant and discard pellet.
8. Proceed to microarray spotting or store lysate at -80°C until microarray spotting.

V. GBL Lysis Buffer:

Grace Buffer formula (GBL Lysis Buffer) was designed to work as a general lysis buffer optimized for printing on ONCYTE film slides to give maximal signal to noise.

1. Mix 0.250 ml each of Lysis Reagent F and protease-free water.
2. Add inhibitor stocks:
 - a. 100 μl 0.5M Sodium Fluoride
 - b. 5 μl 1 M beta-glycerophosphate
 - c. 5 μl 0.2 M Na_3VO_4
 - d. 5 μl 0.2 M PMSF
 - e. 10 μl 0.1 M DTT
 - f. 375 μl water
 - g. Note: This brings volume up to 1ml.
3. Add any additional inhibitors if necessary (optional, see Appendix B).
4. Mix appropriate lysis buffer volume for cells/tissues to be lysed. (1ml/40mg sample or 3×10^6 cells)
5. Vortex for 15 seconds or perform mechanical tissue disruption (if necessary).
6. Incubate for 15 minutes at room temperature.
7. Boil the cell lysate for 10 minutes at 100°C .
8. Centrifuge at 14,000 rpm for 5 min at 4°C .
9. Collect the supernatant and discard pellet.
10. Proceed to microarray spotting or store lysate at -80°C until microarray spotting.

Microarray Printing and Storage

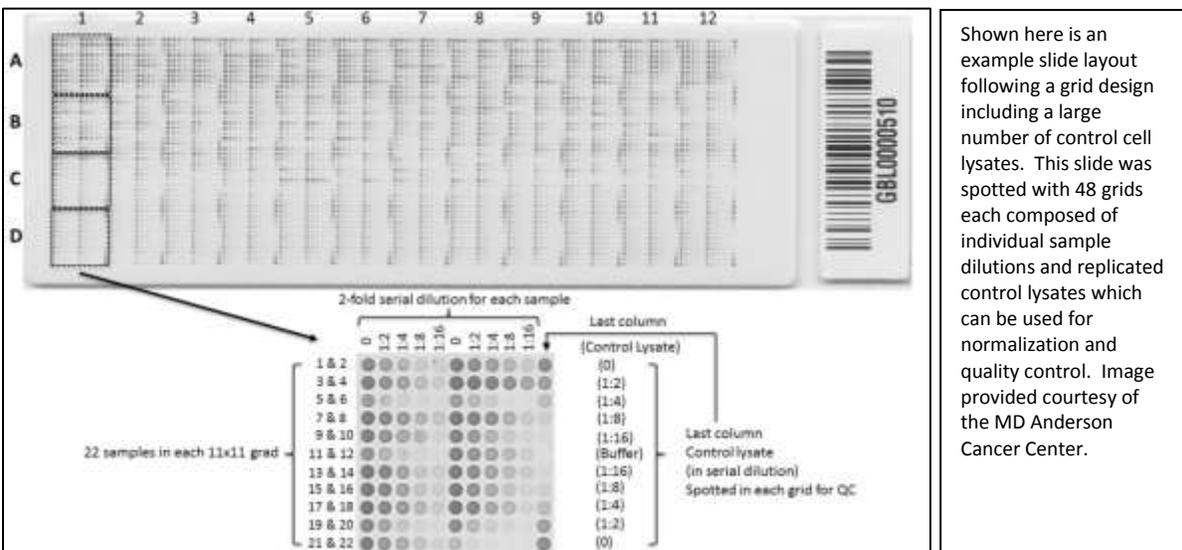
Many parameters for spotting of RPPA will vary depending on the spotter utilized. Environmental factors such as temperature and humidity can affect spot size and morphology and should be controlled for consistent results.

Additionally, the spotting plate layout should be considered at this point with the specific experiment, assay, and microarray printer in mind. Depending on your spotter capabilities, the final slide grid layout will be determined by how samples, dilutions, and controls are laid out in the spotting plate. In addition to layout, appropriate sample dilutions and controls should be determined to ensure optimal data quality. Below is shown an example slide layout including a series of sample and control lysate dilutions.

If lysates were stored frozen prior to printing, precipitation of the spotting buffer components may occur. It is recommended to heat samples to 100°C for 2 minutes prior to performing sample dilutions and spotting. It is NOT recommended to heat samples if they are in the Urea-based lysis buffer.

General Recommendations for Printing and Handling of Slides:

- Do not pre-treat ONCYTE porous nitrocellulose film slides prior to spotting.
- Humidity control (if available) set to 50 – 60%.
- Sample plate lids can be utilized during spotting (if possible with your spotter) to minimize sample evaporation.
- Pre-incubate slides in the spotter at desired humidity for 1 hour prior to printing.
- Allow printed arrays to sit in humidified spotter for 1 hour post-printing.
- Store slides in the slide box provided (light blue GBL box). Other slide boxes and trays may contain materials which can off-gas and significantly impact microarray results.
- Store slides desiccated overnight at 4°C to maximize protein binding before use.
- For long-term storage, store slides desiccated at -20°C to -80°C.



Shown here is an example slide layout following a grid design including a large number of control cell lysates. This slide was spotted with 48 grids each composed of individual sample dilutions and replicated control lysates which can be used for normalization and quality control. Image provided courtesy of the MD Anderson Cancer Center.

RPPA Assay Protocol

Before proceeding, ensure all buffers are diluted to the appropriate working concentrations. Prepare 1X PBST and 1X TBST Wash Buffers from 10X stock solution provided, adding 9 parts protease-free water to 1 part 10X stock solution (See Appendix C). Optional: Prepare The Antigen Retrieval Reagent to 1X in protease-free water. The Blocking Buffers are supplied at 1x concentrations and do not require dilution. All steps may be performed within the well(s) of a ProPlate™ Microarray Chamber, or other slide holder device. During incubations and washes, it is highly recommended to agitate the slides (such as in an orbital shaker) at approximately 50 rpm.

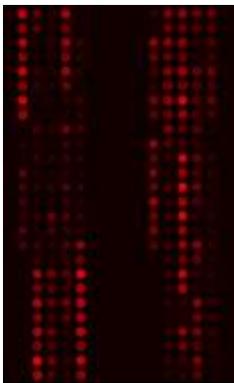
Note: For optimal results, it is VERY important to perform the blocking steps as described.

1. To remove excess spotting buffer salts and detergents prior to beginning assay, wash RPPA slides in fresh deionized water (4 x 15 min) with agitation (this should be performed in staining jar with large wash volumes).
2. Remove slides from the final water wash, attach a ProPlate chamber, and fill wells with fresh deionized water (working with one slide at a time facilitates this process).
3. (Optional) Perform incubation with **Antigen Retrieval Reagent**. This reagent enhances availability of some antigen binding sites through further denaturation.
 - a. Remove slides from water and incubate for 15 min. in 1x Antigen Retrieval Reagent.
 - b. Wash with deionized water (2 x 5 min), completely covering slides.
4. Wash with 1x PBST (2 x 5 min).
5. Incubate with **Blocking Buffer 1** (10 min).
6. Wash with 1x TBST (2 x 5 min).
7. Proceed with additional assay blocking incubations (if necessary) such as biotin and avidin blocking.
8. Incubate with primary antibody. Typically 30-60 minutes is sufficient.
9. Wash with 1x TBST (2 x 5 min. with buffer changes).
10. Perform blocking with **Blocking Buffer 2** (10 – 60 min).
11. Wash with 1x TBST (3 x 5 min. with buffer changes).
12. Incubate with secondary antibody. Typically 30-60 minutes is sufficient.
13. Wash with 1x TBST (2 x 5 min. with buffer change).
14. *OPTION*: Depending on detection method, perform additional assay amplification incubations such as streptavidin-HRP conjugation, Tyramide Signal Amplification, etc.
 - a. Wash with 1x TBST (2 x 5 min. with buffer change) between steps.
 - b. After final incubation, wash with 1 x TBST (3 x 5 min).
15. After all washes in TBST, rinse with fresh deionized water.
16. Allow slides to dry at room temperature for 10 min. prior to data acquisition.

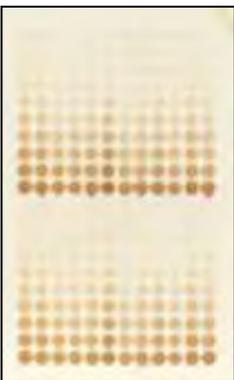
Data Acquisition and Microarray Analysis

There are various detection methods available for data acquisition from RPPA assays. These include colorimetric, fluorescence, and chemiluminescence. The equipment necessary for each detection method will vary and you should consult to each specific manufacturer's instructions for proper use. Sample images from RPPAs with various forms of detection are shown below.

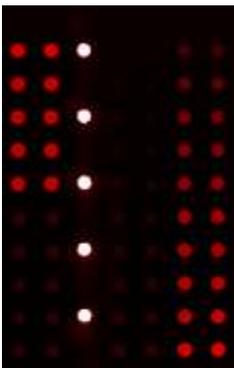
Additionally, each method is subject to variability introduced during the assay or during data acquisition and should be considered when collecting and analyzing data. Inclusion of proper controls during sample spotting will aid in RPPA data analysis and control of assay- and detection-induced variability.



Microarray image generated from an RPPA with fluorescence detection using a conventional fluor (Cy5). The method utilized tyramide signal amplification prior to labeling with Cy5. Microarray was imaged with a Molecular Devices GenePix 4400 microarray scanner with excitation at 635nm.



Microarray image generated from an RPPA with colorimetric detection. The method utilized tyramide signal amplification followed with HRP detection with DAB. Microarray was imaged with a desktop flatbed scanner.



Microarray image generated from an RPPA with fluorescence detection using a near IR dye (IRDye800). The method utilized no signal amplification prior to labeling. Microarray was imaged with an Innopsys InnoScanIR microarray scanner with excitation at 785nm.

Appendices

Appendix A – Kit Reagent Formulas:

<i>Lysis Buffer Reagent A</i> (4x concentrated solution)		
	250 mM	Tris-HCl, pH 6.8
	8%	SDS
	40%	Glycerol
<i>Lysis Buffer Reagent B</i> (4x concentrated solution)		
	150 mM	Tris-HCl, pH 6.8
	8%	SDS
	10 mM	EDTA
	10 mM	EGTA
<i>Lysis Buffer Reagent C</i>		
	100%	TPER
<i>Lysis Buffer Reagent D</i> (2x concentrated solution)		
	100 mM	HEPES, pH 7.4
	2%	Triton X-100
	20%	Glycerol
	2 mM	EGTA
	300 mM	NaCl
	3 mM	MgCl ₂
<i>Lysis Buffer Reagent E</i> (1 of 2)		
	5.4 g	Urea
<i>Lysis Buffer Reagent E</i> (2 of 2, concentrated solution)		
	108 mM	CHAPS
	3.3%	Pharmalytes, pH 8 – 10.5
<i>Lysis Buffer Reagent F</i> (4x concentrated solution)		
		SDS-based GBL Formula
<i>Antigen Retrieval Reagent</i> (10x concentrated solution)		
		Basic GBL Formula
<i>Blocking Buffer 1</i> (1x solution)		
		Super G-based Blocker
<i>Blocking Buffer 2</i>		

(1x solution)		
		Super G Blocking Buffer
<i>10X PBST</i>		
	0.1 M	Sodium Phosphate, pH 7.4
	1.5 M	NaCl
	1%	Tween-20
<i>10X TBST</i>		
	0.5 M	Tris-HCl, pH 7.6
	3 M	NaCl
	1%	Tween-20

Appendix B – Protease and Phosphatase Inhibitor Recipes

Stock Reagent	Weight	Final Volume	Special Preparation Notes:
0.5 M Sodium Fluoride			Sigma S7920
	210 mg	10 ml water	Aliquot into 0.5ml volumes and freeze at -20°C.
0.2 M Sodium Orthovanadate			Sigma S6508
	368 mg	10 ml water	Solution will appear yellow due to color of decavanadate. Adjust pH to 10 and boil solution (solution will turn translucent). Allow to cool, adjust pH to 10 again and boil. Allow to cool. (Repeat until pH remains at 10 and solution remains translucent when cool).
			Aliquot into 0.5ml volumes and freeze at -20°C.
0.1 M Sodium Pyrophosphate			Sigma S6422
	266 mg	10 ml water	Aliquot into 0.5ml volumes and freeze at -20°C.
1M β -Glycerophosphate			Sigma G9422
	2.16 g	10 ml water	Aliquot into 0.5ml volumes and freeze at -20°C.
1 M Dithiothreitol			Sigma 43815
	1.54 g	10 ml water	Aliquot into 0.5ml volumes and freeze at -20°C.

0.2 M Phenylmethylsulfonyl fluoride			Sigma 78830
	348 mg	10 ml isopropanol	Extremely Toxic. Work in a chemical fume hood for all steps.
			Aliquot into 0.5ml volumes and freeze at -20°C.

Inhibitor cocktails may also be purchased from commercial sources. Two recommended inhibitor cocktails are listed below. Working concentrations are 1 tablet per 10 ml lysis 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)

Appendix C – Working Reagent Formulas:

Tris-HCl/SDS/Glycerol Lysis Buffer Formula

(Composed of Lysis Reagents A + B)

50 mM Tris-HCl, pH 6.8

2% SDS

5% Glycerol

2.5 mM EDTA

2.5 mM EGTA

1% β-mercaptoethanol

50 mM Sodium Fluoride

1 mM Na₃VO₄

1 mM DTT

5 mM β-glycerophosphate

1 mM PMSF

Tris-HCl/SDS/Glycerol/TPER Lysis Buffer Formula

(Composed of Lysis Reagents A + C)

62.5 mM Tris-HCl, pH 6.8

2% SDS

10% Glycerol

50% TPER

2.5% β-mercaptoethanol

HEPES/Triton X-100/Glycerol/Tris-HCl/SDS Lysis Buffer Formula

(Composed of Lysis Reagents D + A)

50 mM HEPES, pH 7.4

62.5 mM Tris-HCl, pH 6.8

1% Triton X-100

2% SDS

10% Glycerol

1 mM EGTA

300 mM NaCl

1.5 mM MgCl₂

0.1 M Sodium Fluoride

1 mM Na₃VO₄

10 mM Sodium Pyrophosphate

Urea/CHAPS Lysis Buffer Formula

(Composed of Lysis Reagent E, Parts I + II)

9 M Urea

65 mM CHAPS

2% Pharmalyte, pH 8 – 10.5

65 mM DTT

GBL Lysis Buffer Formula

(Supplied as 4x Lysis Reagent F, dilute to 1x with protease-free water)

1% β-mercaptoethanol

50 mM Sodium Fluoride

1 mM Na₃VO₄

1 mM DTT

5 mM β-glycerophosphate

1 mM PMSF

Antigen Retrieval Reagent (1x solution)

Supplied as 10x concentrated solution

Dilute 1:10 with protease-free water to obtain 1x working solution.

Blocking Buffer 1

Supplied as a 1x solution, ready to use.

Blocking Buffer 2

Supplied as a 1x solution, ready to use.

1x PBST

(Diluted from 10x concentrated solution)

10 mM Sodium Phosphate, pH 7.4

150 mM Sodium Chloride

0.1 % Tween-20
1x TBST
(Diluted from 10x concentrated solution)
50 mM Tris-HCl, pH 7.6
300 mM Sodium Chloride
0.1 % Tween-20