



SH2 Domain Array

User Manual
Cat #: MA3040

Panomics, Inc.

SH2 Domain Array Kit User Manual

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When describing a procedure for publication using this product, we would appreciate it if you would refer to it as the SH2 Domain Array Kit from Panomics

If a paper cites the SH2 Domain Array and is published in a research journal, the lead author(s) may receive a travel stipend for use at a technology conference or tradeshow by sending a copy of the paper to our technical support group at techsupport@panomics.com or via fax at (510) 818-2610.

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Introduction

Array Overview Panomics' SH2 Domain Array includes 38 different human SH2 domains. These arrays are made using the recombinant conserved binding sites of individual SH2 domain proteins fused with glutathione-S-transferase (GST). Proteins are affinity-purified and immobilized onto a membrane. Each SH2 domain on the arrays is spotted in duplicate.

For a complete map and list of the domains included on the array, see Appendix A.

Introduction SH2 Domain Structure & Function

Now that the draft sequence of the human genome is complete, we are faced with myriad new proteins whose functions remain a mystery. An important step toward characterizing the function of a SH2 domain-interacting ligand and/or protein is to identify to which SH2 domain it binds, and hence determine with which signaling pathway it is involved

A key to understanding cellular signal transduction is clarifying how proteins interact with one another. Protein-protein interactions are often mediated by non-catalytic, conserved domains. One of these domains is the SH2 domain.

The SH2 domain was first identified in the retroviral oncoprotein v-FPS as a non-catalytic kinase domain important for phosphotyrosine signaling (1). SH2 domains are defined by conserved region of approximately 100 amino acid residues that is required for multi-protein complex formation (2). While SH2 domains do not have kinase activity, these domains regulate the function of catalytic kinase domains and are involved in mediating tyrosine kinase interactions with cellular substrates. Interaction between proteins with SH2 domains and their binding partners is direct, specific, and phosphotyrosine dependent. These interactions facilitate the recruitment of tyrosine kinase-associated proteins and lead to the activation of downstream signaling cascades (3-5).

Proteins that contain SH2 domains are present in a variety of cellular locations and perform many biochemical functions. For example, SH2 domains can be found in enzymes, adaptor proteins, regulatory subunits of signaling proteins, scaffold proteins, transcription factors and oncogenic proteins. These proteins play critical roles by acting as adaptors between receptors and downstream signaling molecules, transmitting signals within a cell and regulating the kinase activity of specific proteins (3-5). Changes in protein phosphorylation are a major conduit of information for cellular responses, and defects in SH2 domain-dependent signaling are often directly or indirectly shown to be involved in human diseases (5).

Assay WorkFlow Traditional methods for detecting protein-protein interactions, such as co-immunoprecipitation, are arduous and time consuming at best. Panomics offers a convenient tool for identifying interactions between a ligand and SH2 domains that remain folded in active conformations.

With Panomics' SH2 Domain Arrays, you can determine whether your protein of interest binds to multiple SH2 domains—all in one experiment. First, the membrane can be incubated with one of the following three sample inputs for further SH2 domain analysis.

1. Recombinant protein of interest engineered with His-tag
2. Purified protein of interest conjugated to biotin
3. Synthetic phosphotyrosine peptide conjugated to biotin

The samples are incubated with the membrane and if the proteins/ligand of interest has a specific target of an SH2 domain, it will bind to the recombinant SH2 protein that has been spotted on the membrane. The SH2 protein/ligand complex are then further incubated with Streptavidin-HRP or an anti-His HRP detection antibody. The membrane is then incubated with an HRP-based chemiluminescence detection reagents the binding signal will be observed by film or an imaging system.

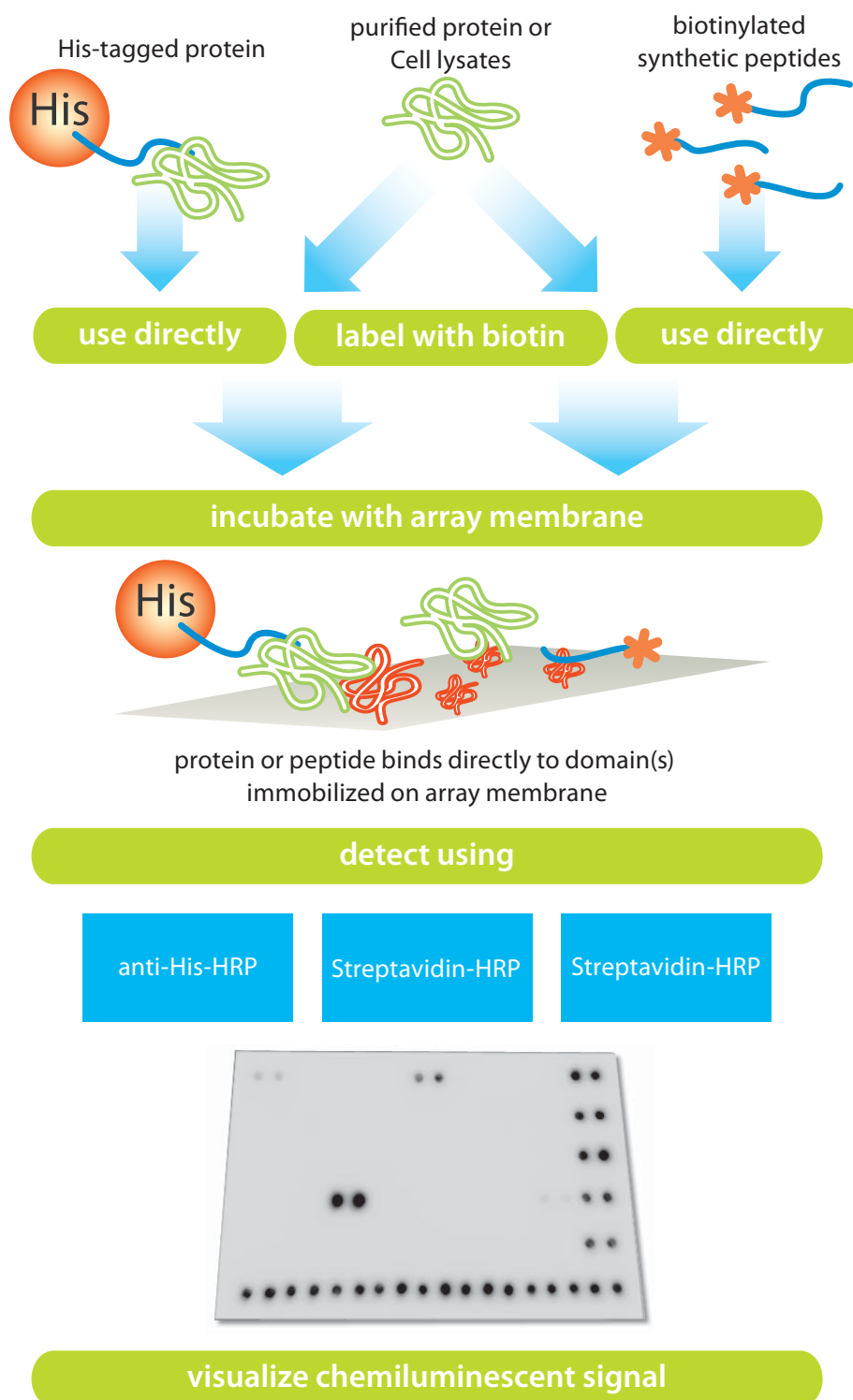


Figure 1: Flow chart of the SH2 Domain Array assay.

Materials

Materials Provided **STORAGE CONDITIONS:** Upon receipt, store the entire kit 4°C until ready for use.

Component	Description
SH2 Domain Array	2 each; 3 cm wide x 6 cm long
1X Blocking Buffer	15 mL
1X Streptavidin-HRP Conjugate	1 mg/mL; 30 µL
10X Wash Buffer (dilute with dH ₂ O)	15 mL
Detection Buffer A	600 µL
Detection Buffer B	600 µL
Four-Well Tray	1 each

Sufficient quantities of each buffer are provided for two assays.

Additional Items Required Required Equipment and Materials Not Provided

Item	Source
Microcentrifuge	Major Laboratory Supplier
Orbital shaker	Major Laboratory Supplier
Pipette, P1000, P200	Major Laboratory Supplier
Plastic sheet protector	Office supply
Film	Hyperfilm™ ECL™ (GE Healthcare, Cat.# RPN1674K) or equivalent
Chemiluminescent Imaging System	Chemiluminescence imaging system (e.g., FluorChem® from Alpha Innotech Corp.)

Overview

General Considerations

With the SH2 Domain Array, we recommend using:

- 1 Biotin-conjugated phosphotyrosine peptide. This method uses streptavidin-HRP for detection, which is included with the kit.

OR

- 2 Biotin-labeled peptide or protein that have been tyrosine phosphorylated *in vivo* or *in vitro*. This method uses streptavidin-HRP for detection, which is included with the kit.

OR

- 3 Proteins that have been tyrosine phosphorylated *in vivo* or *in vitro*. This method requires an antibody specific to the protein or a tag and the appropriate HRP-conjugate.

Handling array membranes:

1. Always use forceps to handle membranes by the edge only.
2. Always place array membranes protein-side up. To do this, use the notched corner (top-right when longer side of membrane is horizontal) for orientation.
3. Never allow array membranes to dry during experiments.
4. Stripping of the array membranes is not recommended.

Incubation:

1. To avoid drying, completely cover membranes with sample or buffer during incubation steps.
2. Perform all incubation and wash steps under gentle rotation.
3. Avoid foaming during incubation steps.

Incubation In this Section, you will incubate the biotin-conjugated tyrosine-phosphorylated peptide, protein or extract with the array membrane. For purified proteins use at a concentration of 5-20 mg/mL. Please Note that the array membranes have a notch at the top right-hand corner for orientation purposes.

Note: *Be sure that the membrane is fully submerged in assay buffer at all times. Never let the membrane dry out.*

- 1.1 Prepare Peptide Probe Mix:
 - 1.1.1 Mix 15 μ L of biotin-conjugated peptide (1.5 ng - 1.5 μ g) with 15 μ L 1 mg/mL Streptavidin-HRP.
 - 1.1.2 Incubate with gentle rocking for 30 min at 4°C.
 - 1.1.3 Add to 5 mL of 1X Blocking Buffer. Store at 4°C until ready to use.
- 1.2 Place each membrane into the provided four-well tray containing 5 mL of 1X Wash Buffer for 30 min. If necessary, trim the membrane to fit the well (Do not cut the protein spots).
- 1.3 Discard the Wash Buffer and add 5 mL of 1X Blocking Buffer. **Make sure that the membrane is fully submerged in buffer.**
- 1.4 Place the tray on a shaker and incubate at room temperature for approximately 1 to 2 hours, or until each membrane appears uniformly wetted and no dry spots are visible.
- 1.5 Remove 1X Blocking Buffer, and briefly rinse membrane with 6 mL of 1X Wash Buffer.
- 1.6 Add the Peptide Probe Mix to the membrane and incubate with gentle shaking for 1-2 hr at room temperature or overnight at 4°C.
- 1.7 After incubation, wash the membrane three times with 6 mL of 1X Wash Buffer for 10 min (each wash) at room temperature.

Detection **Important note:** Do not let the membrane dry out during detection.

- 2.1 Prepare the detection solution immediately before use by mixing equal amounts of Detection Buffers A and B—e.g., 300 μ L of Detection Buffer A and 300 μ L of Detection Buffer B.
- 2.2 Using forceps to hold the cut corner, carefully remove each membrane from its tray. Drain the excess Wash Buffer from the membrane by touching the bottom edge of the membrane against a paper towel.
- 2.3 Place protein- side up by orienting the notch to the top, right-hand corner onto a clean plastic sheet protector or overhead transparency film. Do not use thin plastic cling wraps as these wrinkle and lead to uneven detection across the membrane.

- 2.4 Pipet the Detection Buffers onto the membrane and overlay the membrane with a second plastic as quick as possible. Ensure that substrate is evenly distributed over the membrane with no air bubbles. Incubate at room temperature for 5 min.
- 2.5 Remove excess substrate by gently applying pressure over the top sheet. Using a soft tissue, remove excess substrate that might be remaining on the surface of the sheets. Expose the membranes using either Hyperfilm ECL (2-10 min) or a chemiluminescence imaging system (5-15 min), such as the FluorChem imager from Alpha Innotech Corp. In either case, we recommend that you try several different exposure times.
- 2.6 Obtain quantitative analysis, if desired. If you are using a chemiluminescence imaging system, follow the instructions that are provided with that system's software. If you are using Hyperfilm ECL, you will need to scan the film and use a third party software program to obtain numerical data for comparison.

Troubleshooting Guide

Problem	Cause	Recommendation
Weak or no signal	Ligand or protein is not binding to the SH2 domains.	Ensure that the ligand or protein is tyrosine-phosphorylated.
High background	Antibody concentration is too high.	Further dilute the antibody.
	Nonspecific interactions with antibodies or other reagents used in the assay.	Check signal using a zero standard (i.e. PVDF membrane alone). High background is usually the result of insufficient blocking. Try longer incubation with the blocking buffer.
Uneven background	Membrane dried out during incubation.	Keep the membrane fully submerged in solution during all incubation steps.
	Volume of blocking solution, sample, or antibody is too low.	Increase the volume to make sure that the membrane is fully submerged during incubation.
	Membrane is not evenly covered by detection buffer.	Increase the volume of detection buffer to make sure that the membrane surface is fully covered.
	Air bubbles on membrane surface during detection.	Remove air bubbles from membrane surface.

Appendix A
Schematic
Diagram for SH2
Domain Array I

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A	Abl1		BRDG1		BTK		CRKL		CSK		EAT2		FES		FGR		Pos	
B	Fyn		GRAP		GRB2		GRB14		HCK		LCK		Lyn		MATK		Pos	
C	NCK1		NCK2		P85A-D1		P85A-D2		P85B-D1		P85B-D2		PLCg1-D1		PLCg1-D2		Pos	
D	PTPN11-D1		PTPN11-D2		RASGAP1-D1		RASGAP1-D2		RaLP		SHC1		SHC2		SHC3		Pos	
E	Src		STAP2		TNS		YES		ZAP70-D1		ZAP70-D2		GST				Pos	
F	Pos		Pos		Pos		Pos		Pos		Pos		Pos		Pos		Pos	

Schematic diagram of the SH2 Domain Array I. The proteins on the array are spotted in duplicate at 100 ng. Positive marker has been spotted along the bottom (row F) and in duplicate along the right side (column 17, 18) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.

- References**
1. Sadowski, I. *et al.* (1986) *Mol. Cell. Biol.* 6: 4396-4408.
 2. Koch, C.A. *et al.* (1991) *Science* 252: 668-674.
 3. Pawson, T. *et al.* (2001) *Trends in Cell Biology* 11: 504-511.
 4. Schlessinger, J. and Lemmon, M.A. (2003) *Science's STKE* 1991.
 5. Yaffe, M.B. (2002) *Nature Reviews Molecular Cell Biology* 3: 177-186.

Notes

Contacting Panomics

For ordering information or technical support, contact the appropriate resource provided below according to your geographical location.

Location	U.S. Corporate Headquarters
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