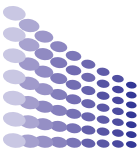
TranSignal™
SH3 Domain Array

Catalog No. MA3010
Product User Manual
Version 1.1



Transcending boundaries...

Panomics, Inc. • 2003 East Bayshore Road • Redwood City, CA 94063 • USA
Tel: 650.216.9736 or 877.726.6642 (PANOMIC) • Fax: 650.216.9790 • **www.panomics.com**

CONTENTS

1. INTRODUCTION 3
2. MATERIALS PROVIDED 6
3. ADDITIONAL MATERIALS REQUIRED 6
4. PREPARE BACTERIAL EXPRESSION CONSTRUCT 7
5. PREPARE BACTERIAL EXTRACTS WITH SH3 LIGAND 7
6. INCUBATION 8
7. WASH & DETECT 8
8. TROUBLESHOOTING 9
9. REFERENCES 10

APPENDIX A: TYPICAL RESULTS OF TRANSIGNAL SH3 DOMAIN ARRAY 11
APPENDIX B: SCHEMATIC DIAGRAM OF THE TRANSIGNAL SH3 DOMAIN ARRAY 11
APPENDIX C: MAP & MCS OF EXPRESSION VECTOR 13

 (UM22302129)

1. INTRODUCTION

One of the keys to understanding cellular signal transduction is clarifying how proteins interact with one another. Protein-protein interactions are often mediated by noncatalytic, conserved domains. One of these domains is the Src homology 3, or SH3, domain (1).

SH3 Domain Structure & Function

First identified as part of the Rous sarcoma oncogene product src, SH3 (src-homology-3) domains play an important role in intercellular communication and intracellular signal transduction. Each SH3 domain is a small, conserved sequence of about 60 amino acids that interacts with proline-rich binding sites. These sites, known as SH3 ligands, contain 6-to-12 residues, with a conserved Pro-Xaa-Xaa-Pro (PXXP) motif (1).

SH3 domains act as part of an adapter molecule, recruiting downstream proteins in a signaling pathway. SH3 domains mediate interactions in many key signaling pathways, including epidermal growth factor receptor signaling (2), cellular localization of cytoplasmic proteins (3), upregulation of GTPase activity of dynamin (4), and activation of phosphatidylinositol 3-kinase in response to IgM crosslinking (5). And SH3 domain activity has been implicated in both cancer and AIDS.

Studying SH3 Domains

As we near completion of the human genome project, new protein identities will emerge as will a growing need to study their functions. One way to characterize protein function is to identify which SH3 domain it binds to and hence unlock which signaling pathway it is involved with. In order to dissect signaling pathways that involve SH3 domains, scientists need a way to determine whether their ligand of interest interacts with SH3 domains—and, specifically, which SH3 domains bind to it. But this requires an assay that allows the SH3 domain to stay folded in its active conformation. Traditional methods for assaying protein-protein interactions, such as co-immunoprecipitation, are arduous and time consuming at best.

Observe protein interactions using all-in-one array system

With the TranSignal™ SH3 Domain Array, you can determine whether your protein of interest binds to one or more of the 38 different SH3 domains—all in one experiment. The assay couldn't be simpler: just express your protein of interest in bacteria and incubate the extract with the TranSignal SH3 Domain Array membrane. The protein interactions literally take place on the array membrane, and you can visualize them using chemiluminescent detection.

We've spotted 38 of the most commonly studied SH3 domains on the TranSignal SH3 Domain Array. For a complete map of the array and list of SH3 domains, see the Appendix.

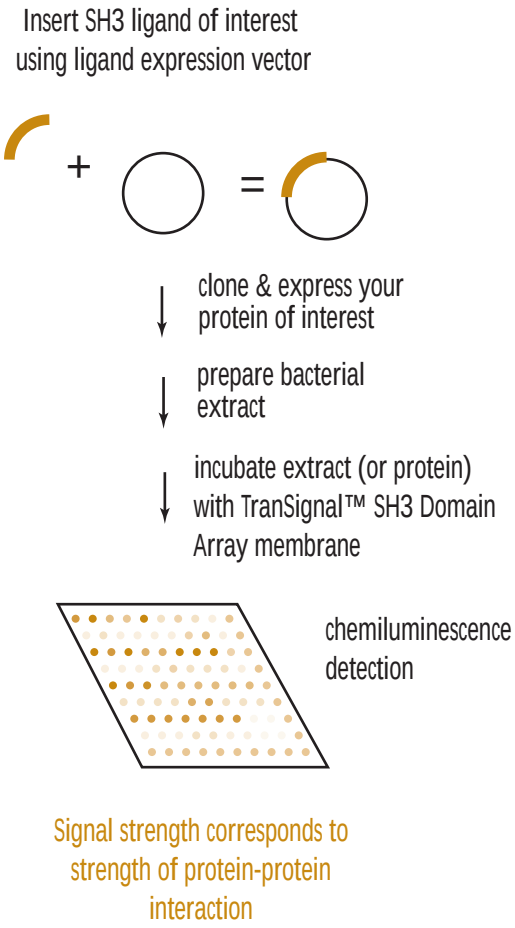


Figure 1. Flow chart of the TranSignal™SH3 Domain Array procedure.

2. MATERIALS PROVIDED

- **TranSignal SH3 Domain Array** (2 each)
- **Positive Control Bacterial Extract** (1.5 ml)
  (From *E.Coli* containing Class Ib Ligand from P13K)
- **pEXP Vector** (Ligand expression vector, 10 µl; 0.08 µg/µl)
- **20X Wash Buffer** (2 x 60 ml)
- **1X Resuspension Buffer** (60 ml)

3. ADDITIONAL MATERIALS REQUIRED

3.1 Reagents and Solutions

- **DH5α Competent Cells** (Gibco BRL, Cat. No. 18265-017)
- **Penta-His Antibody, BSA-free** (Qiagen, Cat. No. 34660)
- **Anti-Mouse IgG (Fab specific) Peroxidase** (Sigma, Cat. No. A-2304)
- **SuperSignal Elisa Pico Chemiluminescent Substrate** (Pierce, Cat. No. 37070)
- **SuperBlock Blocking Buffer** (Pierce, Cat. No. 37545)
- **LB w/Amp** (Teknova, Cat. No. 0181-A100)
  (1.0% Tryptone, 0.5% yeast extract, 1.0% NaCl with 100 µg/ml ampicillin.)
- **IPTG** (Teknova, Cat. No. 13307-50)

3.2 Materials and Equipment

- **Microcentrifuge**
- **Sonicator**
- **Small plastic tray or containers**
- **Shaker**
- **Hyperfilm ECL** (Amersham, Cat.# RPN3114K) or equivalent

OR

- **Chemiluminescence imaging system** (e.g., FluorChem from Alpha Innotech Corp.)

4. PREPARE BACTERIAL EXPRESSION CONSTRUCT

Using the Ligand Expression Vector (provided), insert the SH3 ligand of interest using standard molecular cloning techniques (6). For vector map, see Appendix B. Transform DH5α competent cells with DNA ligation mix as described by the manufacturer's instructions.

5. PREPARE BACTERIAL EXTRACTS WITH SH3 LIGAND

In this section, you will prepare bacterial extract containing your ligand of interest to hybridize with the array membrane (Section 6).

5.1 Inoculate the transformed bacteria in LB/Amp (100 µg/ml) (Section 4).

5.2 Grow bacteria overnight at 37°C with shaking (225 rpm).

5.3 Transfer 40 µl of overnight culture to a tube containing 4 ml of LB/Amp (100 µg/ml).

5.4 Grow bacteria at 37°C until OD600 readings are approx. 0.5–0.8.

5.5 Add 100 µM IPTG.

5.6 Continue to grow for an additional 1.5–2.5 hr.

5.7 Centrifuge cells at 4,000 rpm for 5 min. Decant supernatant.

5.8 Resuspend pellet in 750 µl of ice-cold 1X Resuspension Buffer (provided).

5.9 Lyse cells using a sonicator.

5.10 Centrifuge at 14,000 rpm for 1 min at 4°C.

5.11 Transfer supernatant into a clean microcentrifuge tube.

5.12 Dilute bacterial extract to a final concentration of 0.1 µg/µl in 1X Resuspension Buffer.

5.13 Store on ice until further use. For longer storage, keep at –20°C.

6. INCUBATION

In this Section, you will incubate the bacterial extract containing your SH3 ligand of interest (prepared in Section 5) to the array membrane. Be sure to prepare additional reagents (not included with this kit), as described by the manufacturer.

6.1 Place each membrane into a small tray containing 30 ml of 1X SuperBlock Blocking Buffer (prepare buffer according to manufacturer's instructions).

6.2 Place the tray on shaker and incubate for 30 min at room temperature.

6.3 Remove 1X SuperBlock Blocking Buffer, and briefly rinse membrane two times with 1X Wash Buffer.

6.4 Incubate with 30 ml of diluted bacterial extract (from Step 5.12 or use the Positive Control Extract, provided) at room temperature for 1 hr or 4°C, overnight.

7. WASH & DETECT

7.1 After incubation, wash three times with 40 ml of 1X Wash Buffer for 5 min (each wash).

7.2 Incubate with Penta-His Antibody (1:2000 dilution in 1X Wash Buffer) for 1 hr at room temperature.

7.3 Wash three times with 40 ml of 1X Wash Buffer for 5 min (each wash).

7.4 Incubate with Anti-mouse IgG Peroxidase (1:2000 dilution in 1X Wash Buffer) for 1 hr at room temperature.

7.5 Wash three times with 40 ml of 1X Wash Buffer for 5 min (each wash).

7.6 Detect using SuperSignal Substrate (from Pierce). We recommend 1 ml of working solution per membrane. Expose the membranes using either Hyperfilm ECL or a chemiluminescence imaging system, such as the FluorChem imager from Alpha Innotech Corp. In either case, we recommend that you try several different exposures of varying lengths of time (e.g., 30 sec–5 min).

8. TROUBLESHOOTING

Problem	Cause	Recommendation
• Weak or no signal	Expressed ligand does not have a His-tag	Check construct by DNA sequencing. Ensure that the cloned insert does not contain an internal translational start site.
	His tag is partially hidden	Protein binding may be hindered by a partially hidden His tag. Try using a high concentration (5–10X of the bacterial lysate) or use longer binding time.
	Primary or secondary antibody is no longer working	Check by dot blot to determine if antibodies are working properly.
• High background	Nonspecific interaction 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 the antibody system used. Try using a less sensitive substrate, such as ECL.
	The ligand concentration is too high	Dilute the bacterial lysate (30X).
	The blocking solution is not working properly	Test the blocking solution with western blot or positive control membrane.
	Secondary antibody concentration is too high	Lower the concentration of secondary antibody.
• Uneven background	The volume of primary antibody is too low	Increase the volume to make sure that the membrane is fully covered during incubation.

9. REFERENCES

1. Pawson, T. (1995) Protein modules and signalling networks. *Nature* **373**:573–580.
2. Lowenstein, E.J., Daly, R.J., Batzer, A.G., Li, W., Margolis, B., Lammers, R., Ullrich, A., Skolnik, E.Y., Bar-Sagi, D. and Schlessinger, J. (1992) The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* **70**:431-42.
3. Bar-Sagi, D., Rotin, D., Batzer, A., Mandiyan, V. and Schlessinger, J. (1993) SH3 domains direct cellular localization of signaling molecules. *Cell* **74**:83–91.
4. Gout, I., Dhand, R., Hiles, I.D., Fry, M.J., Panayotou, G., Das, P., Truong, O., Totty, N.F., Hsuan, J., Booker, G.W., et al. (1993) The GTPase dynamin binds to and is activated by a subset of SH3 domains. *Cell* **75**:25–36.
5. Pleiman CM, Hertz WM, Cambier JC. (1994) Activation of phosphatidylinositol-3' kinase by Src-family kinase SH3 binding to the p85 subunit. *Science* **263**:1609–1612.
6. Alexandropoulos, K., Cheng, G., and Baltimore, D. (1995) Proline-rich sequences that bind to Src homology 3 domains with individual specificities. *Proc. Natl. Acad. Sci. USA* **92**:3110–3114.
7. Rickels, R.J., Botfield, M.C., Zhou, X.M., Henry, P.A., Brugge, J.S. and Zoller, M.J. (1995) Phage display selection of ligand residues important for Src homology 3 domain binding specificity. *Proc. Natl. Acad. Sci. USA* **92**:10909–13.
8. Weng, Z., Rickles, R.J., Feng, S., Richard, S., Shaw, A.S., Schreiber, S.L. and Burgge, J.S. (1995) Structure-function analysis of SH3 domains: SH3 binding specificity altered by single amino acid substitutions. *Mol.Cell Bio.* **15**:56327–34.

APPENDIX A: TYPICAL RESULTS OF TRANSIGNAL SH3 DOMAIN ARRAY

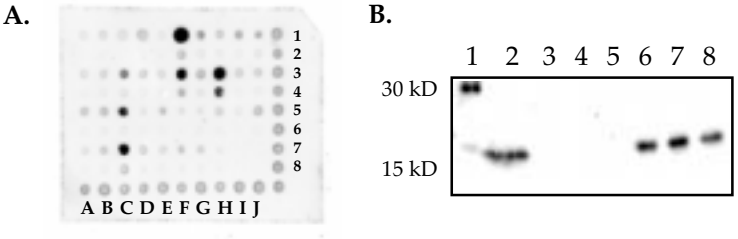


Figure 1. Typical results of TranSignal SH3 Domain Array. Panel A. *Bacterially expressed class Ib SH3 ligand specifically interacts with corresponding SH3 domains. Class Ib encoding sequence was inserted into the Ligand Expression Vector, and DH5a was transformed with the resulting construct. Bacterial extract from the transformed cells was hybridized with the TranSignal SH3 Domain Array, and the image was acquired using FluorChem imager (from Alpha Innotech). Spots with higher intensity indicate higher binding affinity with ligand of interest to SH3 Domain(s).* ***Panel B.*** *Class Ib SH3 ligand specifically "pulls down" its corresponding SH3 Domains. Lane 1: marker. Lane 2: ligand. Lane 3: GST. Lane 4: EMP55. Lane 5: CCB4. Lane 6: c-Src. Lane 7: Lyn. Lane 8: Yes.*

APPENDIX B: SCHEMATIC DIAGRAM OF THE TRANSIGNAL SH3 DOMAIN ARRAY

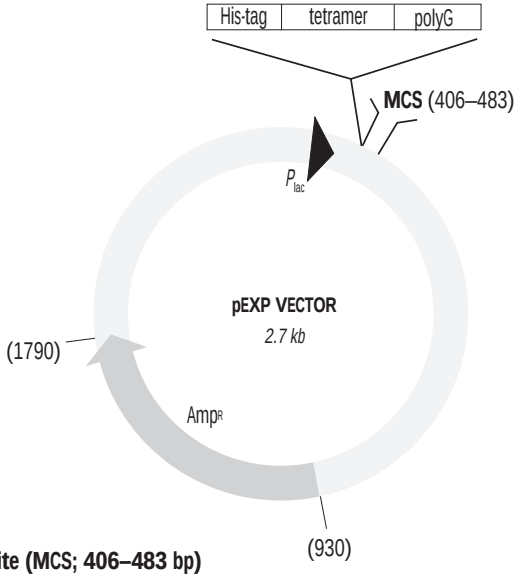
	A	B	C	D	E	F	G	H	I	J	
1	Amphiphysin	CCB4	SPCN	Cortactin	MLPK3	Yes1	Lyn1	SJHUA	Itk	CRK	1
2	Amphiphysin	CCB4	SPCN	Cortactin	MLPK3	Yes1	Lyn1	SJHUA	Itk	CRK	2
3	Dlg2	EMP55	FGR	SLK	Nebulin	c-Src	FYB	Hck	VAV2	NOF	3
4	Dlg2	EMP55	FGR	SLK	Nebulin	c-Src	FYB	Hck	VAV2	NOF	4
5	VAV	NCK(3)	Y124	PEXD	BTK	RasGAP	PSD95	Tim	HS1	Abl(2)	5
6	VAV	NCK(3)	Y124	PEXD	BTK	RasGAP	PSD95	Tim	HS1	Abl(2)	6
7	BLK	Abl(1)	PLCr	Riz	ITSN(2)	ITSN(1)	TXK	GST			7
8	BLK	Abl(1)	PLCr	Riz	ITSN(2)	ITSN(1)	TXK	GST			8
	A	B	C	D	E	F	G	H	I	J	

Figure 2. Schematic diagram of the TranSignal™ SH3 Domain Array. *The proteins on the array are spotted in duplicate: the first row is protein spotted normally, the second row is diluted 1:5. His-tagged ligand has been spotted along the right and bottom sides of the membrane. These spots are intended for alignment. (Note that the notch is at the top, right-hand corner.)*

Table 1. List of SH3 Domains

COORD.	SH3 DOMAINS	FULL NAME
A1, 2	Amphiphysin	Amphiphysin
A3, 4	Dlg2	Discs large homolog 2
A5, 6	VAV	Vav 1 oncogene product
A7, 8	BLK	Beta-lymphocyte specific protein tyrosine kinase
B1, 2	CCB4	Dihydropyridine-sensitive L-type calcium channel beta-4 subunit
B3, 4	EMP55	55K erythrocyte membrane protein
B5, 6	NCK(3)	SH3 domain #3 of the melanoma cDNA encoding a cytoplasmic protein conisisting of the src homology units SH2 and SH3
B7, 8	Abl	Abelson tyrosine kinase, SH3 Domain #1
C1, 2	SPCN	Spectrin alpha chain (non-erythrocytic)
C3, 4	FGR	Cellular Gardner-Rasheed feline sarcoma virus protein
C5, 6	Y124	PAK-interacting exchange factor beta
C7, 8	PLCr	Phospholipase C gamma 1
D1, 2	Cortactin	Cortactin
D3, 4	SLK	Src-like kinase
D5, 6	PEXD	Peroxisomal membrane protein pex13
D7, 8	Riz	Retinoblastoma-associated binding protein
E1, 2	MLPK3	Mixed-lineage protein kinase 3
E3, 4	Nebulin	Nebulin
E5, 6	BTK	Bruton Tyrosine Kinase
E7, 8	ITSN(2)	Intersectin, SH3 Domain #2
F1, 2	Yes1	Yamaguchi sarcoma virus oncogene homolog 1
F3, 4	c-Src	Cellular Rous Sarcoma Virus
F5, 6	RasGAP	GTPase-activating protein
F7, 8	ITSN(1)	Intersectin, SH3 Domain #1
G1, 2	Lyn	Lyn protein non-receptor kinase
G3, 4	FYB	Fyn binding protein
G5, 6	PSD95	Presynaptic density protein 95
G7, 8	TXK	TXK tyrosine kinase
H1, 2	SJHUA	Spectrin alpha chain
H3, 4	Hck	Hemopoietic cell kinase
H5, 6	Tim	Rho guanine nucleotide exchange factor (GEF) 5
H7, 8	GST	Glutathione S-transferase
I1, 2	Itk	Interleukin-2-inducible T-cell kinase
I3, 4	VAV2(2)	Vav 2 oncogene product, SH3 Domain #2
I5, 6	HS1	Hematopoietic specific protein 1
I7, 8	- -	
J1, 2	CRK(2)	Avian sarcoma virus CT10 oncogene homolog, SH3 Domain #2
J3, 4	NOF	Neurite outgrowth factor
J5, 6	Abl(2)	Abl, SH3 Domain #2
J7, 8	- -	

APPENDIX C: MAP & MCS OF EXPRESSION VECTOR



Multiple Cloning Site (MCS; 406–483 bp)

GCA TGC CTG CAG GTC GAC TCT AGA GGA TCC CCG GGT ACC GGT ACT AGT AGA AAA AAT GAG TAA CGG CCG TAC GGG CCC

Sph I *Pst* I *Xba* I *Xma* I *Sma* I *Kpn* I *Age* I *Spe* I *Eag* I *BsiW* I *Apa* I

Description:
The ligand expression vector is designed for use with the TranSignal SH3 Domain Array. Insert your SH3 ligand of interest using standard molecular cloning techniques. Then follow the protocol, as described in Section 5, to prepare bacterial extracts containing your SH3 ligand of interest. Sequence Information for this vector can be downloaded from our web site at www.panomics.com.

NOTES:

NOTES: