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Hypromatrix
The Proteomics Company

Instruction Manual For AntibodyArray™

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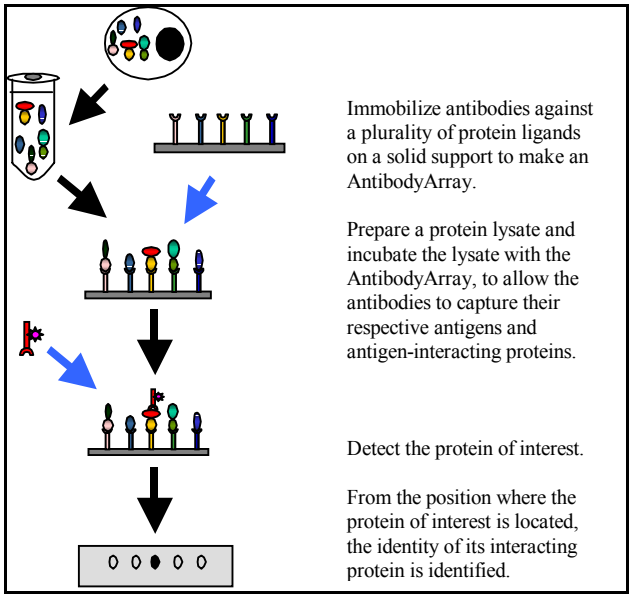
I. INTRODUCTION

AntibodyArray™s contain hundreds of high quality antibodies against well-studied proteins. The antibodies are immobilized on a membrane, each at a predetermined position, and they retain their capabilities of recognizing and capturing antigens as well as antigen-associated proteins. The proteins captured on the array can then be detected by immunoblotting.

AntibodyArray™ offers a high-throughput method for protein studies. It has many applications, including:

1. Examining protein expression profiles
2. Screening protein-protein interactions
3. Studying protein posttranslational modifications

AntibodyArray™s developed by Hypromatrix are especially suitable for screening protein-protein interactions and detecting protein tyrosine phosphorylation.



Overview of the method for screening protein-protein interactions using AntibodyArray™

When AntibodyArray™s are used to detect protein-protein interactions or protein tyrosine phosphorylation under different conditions (e.g., different cell types under the same condition or the same cell type under different conditions), valuable information on changes in protein-protein interaction or protein tyrosine phosphorylation can be obtained.

For further information regarding these applications and other issues, please contact Hypromatrix.

II. LIST OF COMPONENTS

A. AntibodyArray™
Store at 4 °C until use. Good for at least 3 months.

III. ADDITIONAL MATERIALS REQUIRED

The following materials are needed but not supplied:

A. Antibody to the protein of interest

B. Reagents required for antibody-HRP conjugation
EZ-link™ activated peroxidase and antibody labeling kit (Product # 31497ZZ) from Pierce (Rockford, IL).

C. Materials required for antibody biotinylation
EZ-link™ Sulfo-NHS-LC-Biotinylation kit (Product # 21430ZZ) from Pierce (Rockford, IL).

D. Solutions:

1. Blocking solution:
   5% dry milk in TBST (150 mM NaCl, 25 mM Tris, 0.05% Tween-20, pH 7.5).

2. Extraction solution:
   Triton Extraction Solution: 15 mM Tris pH 7.5, 120 mM NaCl, 25 mM KCl, 2 mM EGTA, 2 mM EDTA, 0.1 mM DTT, 0.5% triton X-100, 10 µg/ml leupeptin, 0.5 mM PMSF.

   RIPA buffer: 150 mM NaCl, 1.0% NP-40, 0.5% DOC, 0.1% SDS, 50 mM Tris (pH8.0).

3. Washing buffer:
   TBST (150 mM NaCl, 25 mM Tris, 0.05% Tween-20, pH 7.5).

E. Peroxidase substrate:

1. ECL™ Western blotting detection reagents from Amersham Pharmacia Biotech.

2. NEN™ Western Blot Plus from NEN™ Life Science Products.

IV. METHODS

A. General considerations

1. Before using AntibodyArray™, tests should be done to get information on the quality of the antibody against the protein of interest. The HRP-conjugated antibody should work well in Western blotting and the sensitivity should be comparable to that of unconjugated antibodies.
2. The extraction condition should be optimized to ensure that the protein of interest and its potential interacting proteins are extracted as much as possible from cells and their interactions should not be disturbed.
3. Protein-protein interactions detected by AntibodyArray™ should be further confirmed by other methods, such as co-immunoprecipitation and *in vitro* binding assay.

B. Protocol 1 for screening protein-protein interactions

1. Incubate AntibodyArray™ in blocking solution for at least 1 hour at room temperature with slow shaking. The preferred blocking solution is 5% dry milk in TBST (150 mM NaCl, 25 mM Tris, 0.05% Tween-20, pH 7.5).
2. Incubate AntibodyArray™ with a protein sample (e.g., whole cell extracts) for 2 hours at room temperature with slow shaking.
3. Wash AntibodyArray™ with TBST, 3 x 15 minutes.
4. Incubate the AntibodyArray™ with HRP-conjugated antibody against the protein of interest in TBST for 2 hours at room temperature.
5. Wash AntibodyArray™ with TBST, 3 x 15 minutes.
6. Apply peroxidase substrate and expose to X-ray film.

Note:

1. When the antibodies against the protein of interest are not available, the protein of interest can be expressed as a recombinant fusion protein with a tag, which can be detected by available antibodies. Such tags include but are not limited to His, Myc tag, or GST and green fluorescent protein.
2. Biotinylated primary antibodies may be used. In this case, after incubation with the biotinylated antibody and wash (as in steps 4 and 5), incubate the array with HRP-conjugated avidin for 30-60 minutes, then wash and apply peroxidase substrate.
3. Affinity purified antibodies are recommended. The suggested concentration is 1.5-10 μg/ml for high affinity antibodies or 10-50 μg/ml for lower affinity antibodies.
4. Multiple sites may have positive signal, which suggests that several proteins interact with the protein of interest. These interactions may be direct or indirect, e.g., through a third protein.

C. Protocol 2 for screening protein-protein interactions

In this protocol, the potential interacting proteins and the protein of interest are applied to the array sequentially. That is, the cell lysate (containing potential interacting proteins) is incubated first with AntibodyArray™, and the protein of interest, e.g., as a recombinant protein, is subsequently applied to the array for interactions to occur.

1. Incubate AntibodyArray™ with blocking solution for at least 1 hour at room temperature with slow shaking. The preferred blocking solution is 5% dry milk in TBST (150 mM NaCl, 25 mM Tris, 0.05% Tween-20, pH 7.5).
2. Incubate AntibodyArray™ with a protein sample (e.g., whole cell extracts, nuclear extracts) for 2 hours at room temperature with slow shaking.
3. Wash AntibodyArray™ with TBST, 3 x 15 minutes.
4. Apply the protein of interest, e.g., as a recombinant protein, to the array and incubate for 1-2 hours to allow the interactions to occur.
5. Wash with TBST, 3 x 15 minutes.
6. Incubate the AntibodyArray™ with HRP-conjugated antibody of interest in TBST for 2 hours at room temperature.
7. Wash AntibodyArray™ with TBST, 3 x 15 minutes.
8. Apply peroxidase substrate and expose to X-ray film.

Note:

1. The protein of interest can be expressed in the form of a recombinant protein, such as recombinant GST fusion protein, His tagged protein or GFP fusion proteins. The antibody against the tag (GST, His or GFP) can then be used.
2. The following conjugated antibodies are available from Hypromatrix:
   HRP conjugated anti-His tag antibody
   HRP conjugated anti-GST antibody
   HRP conjugated anti-GFP antibody
   HRP conjugated anti-c-Myc tag antibody
   Biotinylated anti-His tag antibody
   Biotinylated anti-GST antibody

D. Protocol for screening protein tyrosine phosphorylation

1. Incubate AntibodyArray™ with blocking solution for 1 hour at room temperature with slow shaking. The preferred blocking solution is 5% dry milk in TBST (150 mM NaCl, 25 mM Tris, 0.05% Tween-20, pH 7.5).
2. Incubate AntibodyArray™ with a protein sample (e.g., whole cell extracts or nuclear extracts) for 2 hours at room temperature with slow shaking.
3. Wash AntibodyArray™ with TBST, 3 x 15 minutes.
4. Incubate the AntibodyArray™ with HRP- conjugated anti-phosphotyrosine antibody in TBST for 2 hours at room temperature.
5. Wash AntibodyArray™ with TBST, 3 x 15 minutes.
6. Apply peroxidase substrate and expose to X-ray film.

Note:

1. Protein Ser/Thr phosphorylation can be similarly screened by using phospho-Ser/Thr specific antibodies.
2. When screening protein tyrosine phosphorylation HRP-conjugated anti-phosphotyrosine antibodies are available from Hypromatrix.
3. The change in protein tyrosine phosphorylation under two different conditions can be compared.

E. Preparation of whole cell extracts

Care should be taken to make sure that the protein of interest and its potential interacting proteins are extracted as much as possible from cells. For many proteins, such as cytoskeletal proteins, detergents have to be used to solublize them.

For protein-protein interaction studies, mild condition should be used to maintain native protein-protein interactions.

The following is a protocol that can be used to extract most proteins from cultured mammalian cells and maintain protein-protein interactions.

1. Grow cells for two days into fresh confluence in 100 mm petri dish.
2. Remove the cell culture media and wash the cells twice with ice cold Tris saline (50 mM Tris pH 7.5, 150 mM NaCl, 1.5 mM PMSF).
3. Add 1 ml Triton Extraction Solution to cell monolayer and incubate at 4°C for 30 minutes. Triton Extraction Solution contains 15 mM Tris pH 7.5, 120 mM NaCl, 25 mM KCl, 2 mM EGTA, 2 mM EDTA, 0.1 mM DTT, 0.5% triton X-100, 10 μg/ml leupeptin, 0.5 mM PMSF.
4. Pellet cellular debris by centrifugation at maximum speed (14,000rpm) in a desktop centrifuge at 4°C for 15 minutes. Transfer and collect supernatant for AntibodyArray™ assay.
5. Before assay, dilute the protein extract in 5 ml extraction solution containing 1% BSA or 1% dry milk, final concentration should be between 0.5 μg/μl and 5 μg/μl.

Note

1. Radio immunoprecipitation assay buffer (RIPA buffer) can also be used to prepare cell extract for protein-protein interaction screening.
2. For screening protein tyrosine phosphorylation, denaturing buffer can be used. For example, cells can be lysed in 0.6% SDS in PBS and then dilute SDS out with 4 volume of 0.5% Triton in PBS.

F. Preparation of Conjugated Antibody:

Because AntibodyArray™s contain antibodies from several sources, including mouse, rat, rabbit and goat, enzyme-conjugated secondary antibody cannot be used. Enzyme-conjugated or biotinylated primary antibodies have to be used.

HRP Conjugation:

1. Dissolve 1 mg HRP in 0.25 ml of water.
2. Add HRP to 0.1 ml of freshly prepared sodium periodate solution and incubate at room temperature for 20 minutes.Sodium Periodate solution: 0.1 M sodium periodate in 10 mM sodium phosphate (pH7.0).

Sodium Periodate solution: 0.1 M sodium periodate in 10 mM sodium phosphate (pH7.0).

3. Dialyze the above solution overnight at 4°C in 1 mM sodium acetate (pH 4.0).
4. Add the dialyzed HRP to 0.1 ml antibody solution and incubate for 2 hours at room temperature.
   Antibody solution: 1 mg/0.1 ml in 20 mM carbonate buffer (pH9.5).
5. Add 20 μl of sodium borohydride (4 mg/ml in water) and incubate at 4°C for 2 hour
6. Dialyze against PBS.

Antibody Biotinylation

1. Add 20 μl of N-hydroxysuccinimide biotin to 100 μl antibody and incubate at room temperature for 4 hours.
   N-hydroxysuccinide biotin: 10 mg/ml in dimethyl sulfoxide.

   Antibody solution: 1 mg/ml in sodium borate buffer (0.1 M, pH 8.8).
2. Add 5 μl of 1 M NH_4Cl and incubate for 10 minutes at room temperature.
3. Dialyze the antibody solution against PBS.

Note

1. It is important that before using conjugated primary antibodies in AntibodyArray assay, test them in regular Western blotting assay. The conjugated antibody should work well in Western blotting and the sensitivity should be comparable to that of unconjugated antibodies.
2. Other methods can be used. For further information on antibody conjugation, please see Harlow and Lane, 1988, Antibodies, a laboratory manual. Cold Spring Harbor Laboratory Press.
3. Kits from several commercial sources can be used for antibody HRP conjugation and biotinylation.

   EZ-link™ activated peroxidase and antibody labeling kit (Product # 31497ZZ) from Pierce.

   EZ-link™ Sulfo-NHS-LC-Biotinylation kit (Product # 21430ZZ) from Pierce.

V. TROUBLESHOOTING

If the results you obtained is different from what you have expected, use the following guide for troubleshooting. For further help, please contact Hypromatrix.

Observations	Possible Causes	Solutions
No signal	The protein of interest and its interacting proteins are not extracted.	Vary the extraction conditions. Western blot may be performed with the extract to see whether the protein of interest is in the extract.
	Primary Antibody is not sensitive enough.	Use a antibody with higher affinity. Increase the antigen concentration by increasing the amount of protein extract may also partially overcome this problem.
	Conjugation is less satisfactory	Before using conjugated primary antibodies in AntibodyArray assay, test them in regular Western blot assay. The conjugated antibody should work well in Western blotting and the sensitivity should be comparable to that of unconjugated antibodies.
Signal is weak	Dissociation of interacting proteins during incubation.	Decrease the incubation time. Wash for less time.
Too much background	Blocking is not complete	Block the array overnight.
	Antibodies contain reactivities to some components in blocking reagents	Pre-incubate the antibodies with the blocking reagents. Add blocking reagents in Washing solution.
Too many positive spots	Antibody used contains non-specific activities, such as reactivities against all mouse or rabbit antibodies.	Further clean the antibody. If the antibody is not affinity purified, affinity-purify it.

VI. SUGGESTIONS FOR ADDITIONAL EXPERIMENTS

Hypromatrix suggests the following steps to confirm the results obtained with the first screening.

A: Record the positive spots
Carefully analyze the results. Sometimes, a weak positive spot may be real.

B: Repeat the experiment
The positive result from the initial screening should be repeated and confirmed under different conditions. A convenient and economical way to do this is to repeat the experiment using Custom AntibodyArray™s that contain all the antibodies giving positive results. Such customized arrays can be ordered from Hypromatrix.

At least two conditions should be used:

1. Repeat the experiment under the exact condition used in the initial screening.
2. Perform the assay without protein lysates. This will eliminate false positives that result from direct binding between HRP-conjugated detecting antibodies and membrane-bound capturing antibodies.

Other conditions may also be desired. For example, assays can be performed with protein lysates prepared under conditions that the expected interactions will be unlikely to occur. Such conditions include but are not limited to using protein lysates that does not have the protein of interest; preparing lysates under denaturing conditions that disrupt protein-protein interactions. This will ensure that positive signal is not due to direct binding of the same protein by both the capture antibodies and the detecting antibodies.

Carefully compare the results obtained from each condition. Only the antibodies that give positive signals under correct conditions but not under control conditions should be confirmed in additional experiments. Abandon those spots that give signals under control conditions.

C: Confirm the positive results by co-immunoprecipitation.
The results should also be confirmed by other methods, such as immunoprecipitation and *in vitro* protein binding assays. To do this, select the antibodies that give most consistent and reliable results, and perform co-immunoprecipitations with those antibodies. We suggest that individual antibodies used in AntibodyArrays™ from Hypromatrix be used first; then additional antibodies from other sources are used for confirmation.

In many situations AntibodyArray™ assay is more sensitive than co-immunoprecipitation. This is partially due to the fact that antibodies are immobilized at high concentrations on the membrane; and the high antibody density significantly increases the binding of antigens. In addition, because both capturing and detecting antibodies have to bind the same protein complex in order to produce a signal, the chance for false positive result is lower than immunoassays that only require one antibody binding.

Therefore, when a novel interaction identified by AntibodyArray assays is unable to be demonstrated by standard co-immunoprecipitation, it is still quite likely that the interaction is a real one.

D: Additional experiments that may be used for confirmation.
These include in vitro binding assays with recombinant proteins and double immunostaining that can reveal cellular co-localizations of two interacting proteins. In vitro binding is particularly helpful because the assay can tell whether the protein-protein

interaction demonstrated in AntibodyArray™ and co-immunoprecipitation is direct or indirect binding. In addition, some protein interactions that are difficult to demonstrate by co-immunoprecipitation can be revealed by *in vitro* bindings, particularly when right domains of the involved proteins are used in the assays.

VII. REFERENCES

Fields S, Song O (1989) A novel genetic system to detect protein-protein interactions. *Nature* 20; 340(6230):245-6.

Ge, H. (2000) UPA, a universal protein array system for quantitative detection of protein-protein, protein-DNA, protein-RNA and protein-ligand interactions. *Nucleic Acids Research,* Vol. 28:e3.

Harlow and Lane, 1988, Antibodies, a laboratory manual. Cold Spring Harbor Laboratory Press.

Wang, Y., Wu, T.R., Cai, S., Welte, T., and Chin, Y.E. (2000) Stat1 as a component of tumor necrosis factor alpha receptor 1-TRADD signaling complex to inhibit NF-kappaB activation. *Mol. Cell Biol.* 20(13), 4505-12.

APPENDIX I. ANTIBODY LIST BY POSITIONS

A. Signal Transduction AntibodyArray™
Updated: 3-20-2003

	A	B	C	D	E	F	G	H	I	J
1	14-3-3	c-Abl	ACINUS	ACINUS-p23	AIF	Akt 1/2	ALK	Amphiphysin	Bin 1	Ankyrin
2	Bad	Bak	Bax	BOK	NBK	Bag-1	Bcl-2	Bcl-w	Bcl-xS/L	A1
3	BRCA2	Brk	Brm	Btk	C/EBP beta	E-Cadherin	N-Cadherin	VE-Cadherin	Pan-Cadherin	BLCAM
4	Caspase6	Caspase7	Caspase8	Caspase9	Caspase10	a-Catenin	b-Catenin	r-catenin	c-Cbl	CBP
5	Cdk1 / Cdc2	Cdk2	Cdk4	Cdk6	Chk	C-IAP1	C-IAP2	CIDE-A	CIDE-B	Clathrin
6	Cyclin A	Cyclin B	Cyclin D3	Cyclin E	Cyclin H	cytochrome	DAXX	DCC	Desmoglein	DFF45 / ICAD
7	E2F1	EGFR	p-EGFR	Egr-1	Egr-2	Egr-3	Elongin A	EphA1	EphA4	EphB1
8	FAF-1	FAK	Fas	FasL	FAST	FGFR1	FGFR2	FGFR3	FGFR4	FHIT
9	GATA-1	GATA-2	GATA-3	G-CSF R	gp130	Granzyme	GRB2	GRB7	GRB14	GRK 2
10	IEXs/l	IFN-a R a	IFN-g R a	IL 1 Rl	IL 2 R alpha	IL 2 R, beta	IL 2 gamma	IL 3	IL4R a	ING1-p33
11	ISGF3 gamma	Jak1	Jak2	Jak3	JNK1,2,3	p-JNK1,2,3	c-Jun	p-c-Jun	KAP	c-Kit
12	MEF2	MEK1	MEKK1	MEKK2	Menin	Met	MGMT	MMp-3	MMp-9	Mos
13	Ikappa B-a	p-Ikappa B-a	Ikappa B-b	Ikappa B-r	Ikappa B-e	Ikappa B kinase a	Ikappa B kinase b	Nibrin	NIK	nip1
14	p19Skp1	p21WAF1 / CIP1	p27	p35	p38 MAPK	p-p38	p45 skp2	p53	p55 CDC	p57 (Kip2)
15	PDGF	PDGF Receptor b	Phospholipase Cr	Phospholipase D	PI3kinase	PKC α,β,χ	Polo-like kinase	PP1,2A,2B,PPX	pp120	PSD-95
16	RACK1	Rad51	Rad52	c-Raf-1	p-c-Raf-1	RAIDD	RalA	Ran BP-1	Rap1	Rap2
17	Rel B	Ret	Rho A	RICK	RIP	Ron alpha	Rsk-1	Sam68	E-selectin	L-Selectin
18	sp1	sp2	Blk	c-Fgr	fyn	Lck	Lyn	c-Src	Yes	SRF
19	SURVIVIN	Syk	Syntaxin6	TANK	TCR alpha	TCR beta	TDAG51	TGFb R1	TGFb R2	Thyroid R a1
20	TRAF5	TRAF6	TRAIL	Trk A, B, C	TSG101	TTK	Tuberin	Tyk2	VASP	Vav

A. Signal Transduction AntibodyArray™ (continued)
Updated: 3-20-2003

	K	L	M	N	O	P	Q	R	S	T
1	Annexin VI	ANT	Apaf1	APC	ARC	ATF-2	p-ATF-2	Ax1	B7-1	B7-2
2	Mcl-1	Bcl-3	Bcl-6	BID	Bim	BM28	BMX	BAP1	BARD1	BRCA1
3	HCAM	ICAM-1	PECAM-1	VCAM-1	CAS	Caspase1	Caspase2	Caspase3	Caspase4	Caspase5
4	CD 3 epsilon	CD27	CD28	CD30	CD40	CD45	Cdc6	Cdc25A	Cdc34	CDC42GAP
5	Clusterin	Connexin 32	Connexin 43	Cortactin	CPAN	CREB	CREM-1	Ctk	Csk	CUL-1
6	DIVA	DMBT1	DMC1	DcR1	DcR2	DR3	DR4	DR5	Dynamin	Dynamin II
7	eps8	erbB2	erbB3	erbB4	ERK1	ERK2	Estrogen R a	Ets-1/2	Ezrin	FADD
8	FLASH	FLIPs/l	Flt-3/2	Flt-4	c-Fos	Frizzled	GADD34	GADD45	GADD153	GAK
9	GSK-3 alpha	HDAC1	hdlg	Ne-dlg	hILP	Hrk	HS1	HSP-70	ICSBP	Id1
10	Insulin R b	Integrin-alphal	Integrin a 5	Integrin a V	Integrin b 1	Integrin b 3	IRAK	IRF1	IRF2	IRS-1
11	L1	LGI1	LIFR	Mad-1	MAD2	Maspin	Max	MDA-7	MDM2	Mdr
12	Myc (c-Myc)	MyoD	NCK	NF-1	NFIGRP	Nf2	NFATC	NF-kappa B 50	NF-kappa B 52	NF-kappa B p65
13	nip2	nip3	eNOS	iNOS	nNOS	Notch	Ntk	Nurr 1	Ob Receptor	p16
14	P63	P73	p130Cas	p300	Patched	PAR-4	PARP	Pax-5	Paxillin	PCNA
15	PTEN	SH-PTP	PTP1B	SH-PTP2	PYK2	Rabl A	Rab3	Rab5	Rab11	Rac1
16	RAR r	RXR a,b,r	Ras	Ras-GAP	Rb (p107)	Rb (p110)	Rb2 (130)	RBBP	Rbx1 & 2	c-Rel
17	P-Selectin	SHC	SHIP	Sik	SIVA	Smad (1/2/3)	Smad 4	SOCS-1	SODD	Sos1/2
18	STAM	Stat1	p-Stat1	Stat2	Stat3	p-Stat3	Stat4	Stat5a	Stat5b	Stat6
19	TIA-1	TIAR	TNFR1	TNFR2	TOSO	TRADD	TRAF1	TRAF2	TRAF3	TRAF4
20	VDAC1	VDR	VEGFR1	VEGFR2	VHL	WT	XRCC4	YY1	ZAP70 Kinase	ZO-1

B. Apoptosis AntibodyArray™
Updated: 6-19-2003

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	ACINUS-L/S	ACINUS-p23	AIF	Bin 1	ANT	Apaf1	APC	ARC	Bad	Bak	Bax	BOK	NBK	Bag-1	Bcl-2
2	Bcl-w	Bcl-xS/L	A1	Mcl-1	BID	Bim	BAP1	BARD1	BRCA1	BRCA2	CAS	Caspase1	Caspase2	Caspase3	Caspase4
3	Caspase5	Caspase6	Caspase7	Caspase8	Caspase9	Caspase10	CD27	CD30	CD40	C-IAP1	C-IAP2	CIDE-A	CIDE-B	Clusterin	CPAN
4	cytochrome C	DAXX	DCC	DFF45/ICAD	DIVA	DMBT1	DMC1	DcR1 (TRID)	DcR2	DR3	DR4	DR5	Elongin A	FADD	FAF-1
5	Fas/CD95/ APO-1	Fas Ligand	FAST	FHIT	FLASH	FLIPs/l	GADD34	GADD45	GADD153	Granzyme B	hDLG	Ne-dlg	hILP	Hrk	IEXs/l
6	ING1-p33	Jak1	Jak2	Jak3	JNK1,2,3	p-JNK1,2,3	LGI1	Maspin	MDA-7	MDM2	MEF2	MEK1	MEKK1	MEKK2	Menin
7	MGMT	Mye (c-Mye)	NF-1	NF1GRP	Nf2	NF-kappa B 50	NF-kappa B 52	NF-kappa B p65	Ikappa B-a	Ikappa B-b	IKKa	IKKb	Nibrin	NIK	nip1
8	nip2	nip3	p53	P63 (KET)	P73	PAR-4	PARP	PSD-95	PTEN	Rad51	Rad52	RAIDD	Rb p107	Rb (p110)	RbP130 (Rb2)
9	RBBP (RbAp46)	Rel (c-Rel)	RICK	RIP	SIVA	SODD	Stat1	SURVIVIN	TANK	TDAG51	TIA-1	TIAR	TNFR1	TNFR2	TOSO
10	TRADD	TRAF1	TRAF2	TRAF3	TRAF4	TRAF5	TRAF6	TRAIL	TrkA, B, C	TSG101	Tuberin	Tyk2	VDAC1	VHL	XRCC4

C. Cell Cycle AntibodyArray™
Updated: 09-20-2003

	A	B	C	D	E	F	G	H	I	J
1	BM28	BRCA1	BRCA2	Brm	Cdc6	Cdc25A	Cdc34	Cdk1/ Cdc2	Cdk2	Cdk4
2	Cdk6	Chk	CUL-1	Cyclin A	Cyclin B	Cyclin D3	Cyclin E	Cyclin H	DMC1	E2F1
3	GADD34	GADD45	GADD153	GAK	Hdlg	Nc-dlg	ING1-p33	KAP	MAD2	Mos
4	NF-1	NF-kappa B, p65	Ikappa b□	Nibrin	p16	p19Skp1	p21WAF1/CIP1	p27	p35	p45 skp2
5	p53	p55 CDC	p57	P63	P73	PCNA	Polo-like kinase	Rad51	Rad52	Rb (p107)
6	Rb (p110)	RbP130 (Rb2)	RBBP	Rbx1 & 2	Sam68	Stat1	p-Stat1	Stat3	TTK	XRCC4

A. Trial AntibodyArray™
Updated: 3-20-2003

	A	B	C	D	E	F	G	H	I	J
1	14-3-3	Akt 1/2	Axl	E-Cadherin	ICAM-1	β-Catenin	EGFR	Egr-1	EphA1	erb2 (Neu)
2	ERK1	Estrogen R α	Ets-1/2	Ezrin	FAK	FGFR1 (Flg)	FGFR2 (Bek)	Flt-3/2	c-Fos	gp130
3	GRB7	GSK-3 α	HSP-70	IFN-a R α	IL 2 R α	Insulin R β	Integrin α V	Integrin β 3	IRAK	c-Jun
4	c-Kit	eNOS	p38 MAPK	p130Cas	Pax-5	PDGF Receptor α	Phospholipase Cγ	PI3kinase P85	PKC	pp120
5	PTP1 (SH)	PTP1B	PYK2/CAK β	Rab1A	c-Raf-1	RXR α,β,γ	Ras-GAP	Rel B	Rho A	Smad1 (1/2/3)
6	fyn	Src	Yes	Stat5a	Stat6	Syk	TCR α	VEGFR1	YY1	ZAP70 Kinase

APPENDIX II. PRODUCTS FROM HYPROMATRIX, INC

A. AntibodyArray™s:

1. Signal Transduction AntibodyArray™
   Catalog Number HM3000

2. Apoptosis AntibodyArray™
   Catalog Number HM4000

3. Cell Cycle AntibodyArray™
   Catalog Number HM5000

4. Custom AntibodyArray™
   Catalog Number HM6000

B. Antibodies

1. HRP-conjugated antibodies

a. anti-GST
b. anti-GFP
c. anti-6XHis
d. anti-Myc-tag
e. anti-phosphotyrosine
f. HRP-avidin

and more…

2. Primary antibodies

Hypromatrix offers a variety of high quality antibodies. For a complete list of antibodies and their specificities, please visit our web site at www.hypromatrix.com