Genemed Biotechnologies, Inc.

コスモ・バイオ株式会社

PRODUCT ANALYSIS CERTIFICATE

Apoptosis Profiler Array Nonradioactive System

FOR RESEARCH USE ONLY

CATALOG #: 05-102 **SIZE: 1 KIT**

Description: The Apoptosis Profiler™ Array Nonradioactive System employs digoxigenin (DIG)-11-dUTP-labeled cDNA and chemiluminescent detection. Each Apoptosis Profiler Array contains 279 human genes, which have been spotted onto a positively charged nylon membrane. The spots on the array consists of popular and well-studied genes that have been implicated in cell cycle regulation and apoptosis. Enough reagents are provided for four hybridizations.

Package Contents

Profiler Array and Hybridization Kit:

2	Apoptosis Profiler Arrays
25 ml	Hybridization Buffer
60 ml	20X SSC
60 ml	10% SDS

Profiler Array cDNA Synthesis Kit:

10 µg	Control K562 Total RNA
10 µl	Reverse Transcriptase
15 µl	Random Primer Mix
15 µl	Probe Mix
50 µl	100mM DTT
100 µl	5X RT Buffer
100 µl	dH20

Profiler Array Chemiluminescence Detection Kit:

15 ml	CDP-*Star* Substrate
15 ml	Alk-Phosphatase Conjugate
30 ml	5X Blocking Buffer
50 ml	10X Antibody Wash Buffer I
50 ml	10X Antibody Wash Buffer II
30 ml	10X Detection Buffer

Additional Materials

1	Profiler Grid
1	Product User Manual

Storage: Store the Profiler Array and Hybridization Kit at room temperature, Profiler Array cDNA synthesis Kit at –20°C, and Profiler Array Chemiluminescence Kit at 4°C.

Shelf Life: 1 year from date of receipt under proper storage conditions.

Shipping Conditions: Blue ice (4°C) or room temperature.

Quality Control: A sample of this kit was tested according to the user manual. cDNA probes were prepared from Control K562 Total RNA.

(PAC0090)

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Apoptosis Profiler Array Schematic

	Kinases and Phosphatases					Cell cycle		Nuclear Proteins			Cytokines, secreted factors and their receptors						TNK/TNFR family		Intracellular mediators		Caspases, proteases, nucleases, and inhibitors		Bcl-2 Family		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	A-Raf-1	CSNK1A1	INPP5D	MEKK3	PKCβ1	Bub1	Cyclin B1	AP-2α	hsRAD51	NF-AT4	c-mpl	FGF-3	IK	IL-16	IL-20	IL-6R	4-1BB	CD30L	ASC	Fodrin β	Apaf-1	Casp. 13	Bad	BHRF1	A
B	ACP5	DAPK	JAK1	MEKK4	PKCδ	Bub2	Cyclin C	B-myb	ICSBP1	NF-AT5	CD4	FGF-4	IL-10	IL-17	IL-21	IL-6ST	4-1BB Ligand	CD40	B-CATENIN	GPX1	AP14	Casp. 2	BAG1	BID	B
C	AKT1	DNA-PKcs	JAK2	MEKK5	PKCγ	Cdc2	Cyclin D1	BBp53	IKB	NFKB1	CNTF	FGFr	IL-10Rα	IL-17β	IL-21R	IL-7	AP0-3	CD40L	CBL	GSTM 1	ARC	Casp. 3	Bak	BIK	C
D	AKT2	DRAK1	JAK3	MKK4	PLK1	Cdk2	Cyclin E	c-fos	IRF1	NFKB2	CNTFR	FLT-1	IL-10Rβ	IL-17βR	IL-22	IL-7R	APO3L	DR6	CBL-B	GSTT 1	BIRC6	Casp. 4	Baxa	BimEL	D
E	ATM	DRAK2	JNK2	MKP-2	PPP1CB	Cdk3	Cyclin F	c-jun	IRF2	NuMa	CSF-1	GCSF	IL-11	IL-17R	IL-22R	IL-8	CD27	Fas	CRADD	HSF1	BIRC7	Casp. 5	bcl 6	BNIP-1	E
F	c-abl	ERK	JNK3α1	MLK	PPP2CA	Cdk4	Cyclin G1	c-myb	IRF4	P53	EGF	GMCSF	IL-11Rα	IL-18	IL-2Rα	IL-8Rα	CD27L	Fas-Ligand	Cryab	iNOS	CAD	Casp. 6	Bcl-2	BNIP-2	F
G	c-fyn	FAK	JNK3α2	MNK2	PPP2R5A	Cdk5	GADD-45	c-myc	JunB	PARP	EGFR	IFNα	IL-12 p35	IL-18BP	IL-2Rβ	IL-8Rβ	CD30	NGF-2	Cyto C1	MADD	Casp. 1	Casp. 7	Bcl-xL	BNIP-3	G
H	c-lck	FAP	MAPKAPK2	MYT1	PYK2	Cdk6	p15	c-REL	JunD	PU. 1	EPO	IFNαR1	IL-12 p40	IL-18R1	IL-2Rγ	IL-9	PDGFR	NGFB	DAXX	MAP4	Casp. 10	Casp. 8	Bcl2-b	BOK	H
I	c-lyn	FRP1	MAPKAPK5	NIK	RAF1	Cdk7	p16	CREB1	I-myc	RAN	EPOR	IFNαR2	IL-12Rβ1	IL-19	IL-3	IL-9R	SCF	OPG	DDX1	Mdm2	TRADD	Casp. 9	Bcl2-w	Hrk	I
J	c-src	GCK	MEK1	p70S6K	RIP3	Cdk8	p18	CREB2	Lamin A	Rb(p110)	FGF-1	IFNβ	IL-12Rβ2	IL-1α	IL-3Rα	Insulin	TGF-α	Ox40	FADD	PS1	TRAF1	CrmA	Bfl-1	MCL-1	J
K	Calcineurin A	GPRK6	MEK2	PAK1	RIPK1	CHES1	p19	E1A	Lamin B1	Rb2(p130)	FGF-2	IFNγ	IL-13	IL-1β	IL-4	InsulinR	TGF-β	Ox40L	Fodrin α	RasGAP	TRAF3	DFFA	hILP	Ubiq.	K
L	Calcineurin B	GSK3B	MEK3	PFTK1	RIPK2	Cyclin A1	p27	E2F	large T ag	RelA	Stat-4	IFNγR1	IL-13Rα1	IL-1R1	IL-4Rα	ITRAP	TGF-β-R1	p75 NGFR	TRAIL R1	Sentrin	TRAF4	DFFB	I-FLICE	Ubiq.	L
M	CAMK2B	HPK	MEK5	PI3K	RPS6KA6	TAK1	p57	Ets-1	mad	RelB	Stat-5a	IFNγR2	IL-13Rα2	IL-1R2	IL-5	KIT	TGF-β-R2	RANKL	TRAIL R2	SOD1	TRAF5	Granzyme A	MMP1	beta-actin	M
N	CAMK2D	1KKα	MEK6	PITSLARE A1	SAPK4	TIE-1	PA2G4	Ets-2	max	Stat-1	Stat-6	IGF-1	IL-14	IL-1RL1	IL-5Rα	PDGF-α	TGF-β-R3	TNF	TRAIL R3	TAB1	TRAF6	Granzyme B	NAIP	beta-actin	N
O	CD45	1KKβ	MEKK1	PKA	SPHK1	TIE2 (TEK)	PCNA	fos B	n-myc	Stat-2	TR3	IGF-2	IL-15	IL-1RL2	IL-6	PDGF-β	TPO	TNF-R1	TRAIL R4	TANK	TRIP	h IAP 1	Perforin	beta-actin	O
P	CHEK 1	1KKγ	MEKK2	PKCα	SPHK2	WEE 1	Ubiq.	hnRNPC	NF-AT1b	Stat-3	WAF1	IGFBP1	IL-15Rα	IL-2	Ubiq.	Ubiq.	VEGF	TNF-R2	TRAIL	TNFAIP 3	TRPM-2	h IAP 2	TIMP1	beta-actin	P
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	

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Apoptosis Profiler Gene List

CONTROLS	Coordinates	Genbank#
beta-actin	M24, N24, O24, P24	X00351, J00074, M10278
Ubiquitin	K24, L24, P7, 15 & 16	M26880

The following gene list is ordered alphabetically for your convenience.

Gene	Coord.	Genbank #	Gene name
4-1BB	A17	UO3397	Human receptor protein 4-1BB mRNA, complete cds.
4-1BB Ligand	B17	U03398	Human receptor 4-1BB ligand mRNA, complete cds.
ACP5	B1	NM_001611	Homo sapiens acid phosphatase 5, tartrate resistant (ACP5), mRNA.
Akt1	C1	NM_005163	Human v-akt murine thymoma viral oncogene homolog 1 (AKT1)
Akt2	D1	NM_001626	Homo sapiens v-akt murine thymoma viral oncogene homolog 2 (AKT2)
AP-2a	A8	NM_003220	Human transcription factor AP-2 alpha (activating enhancer-binding protein 2 alpha) (TFAP2A) gene
APAF-1	A21	AF013263	Human apoptotic protease activating factor 1 (Apaf-1) gene
API4 (BIRC5)	B21	XM_008623	Homo sapiens baculoviral IAP repeat-containing 5 (survivin) (BIRC5), mRNA
Apo-3	C17	U74611	Human Apo-3 mRNA, complete cds.
Apo-3 Ligand (TWEAK)	D17	AF055872	Homo sapiens Apo3/DR3 ligand (APO3L) mRNA, complete cds.
ARC	C21	AF043244	Homo sapiens apoptosis repressor ARC (ARC) mRNA, complete cds.
ASC	A19	AB023416	Homo sapiens ASC mRNA for apoptosis-associated speck-like protein containing a CARD, complete cds.
ATM	E1	NM_000051	Homo sapiens ataxia telangiectasia mutated (includes complementation groups A, C and D) (ATM), mRNA.
b-catenin	B19	Z19054	Human beta catenin gene
b-myb	B8	X13293	Human B myb oncogene
Bad	A23	AF031523	Human bcl-xL/bcl-2 associated death promoter (BAD) gene
BAG1	B23	NM_004323	Homo sapiens BCL2-associated athanogene (BAG1), mRNA.
BAK	C23	U16811	Human Bak mRNA
Bax-a	D23	L22473	Human Bax alpha gene
BBP53 (TP53BP2)	C8	NM_005426	Homo sapiens tumor protein p53-binding protein, 2 (TP53BP2), mRNA.
bcl-6	E23	U00115	Human zinc-finger protein (bcl-6) mRNA, complete cds.
Bcl-xL	G23	Z23115	Human bcl-xL gene
Bcl2	F23	M14745	Human bcl-2 mRNA
Bcl2-b	H23	M13995	Human B cell proto-oncogene
Bcl2-w	I23	U59747	Human Bcl-w (bcl-w) mRNA
Bfl-1	J23	U27467	Human Bcl-2 related (Bfl-1) mRNA, complete cds.
BHRF1	A24	AF120456	Herpesvirus papio Bcl-2 homolog (BHRF1) gene, complete cds.
BID	B24	NM_001196	Homo sapiens BH3 interacting domain death agonist (BID) mRNA
BIK	C24	U34584	Human Bcl-2 interacting killer (BIK) mRNA
BimEL	D24	AF032457	Homo sapiens BimEL mRNA
BIRC6	D21	NM_016252	Homo sapiens baculoviral IAP repeat-containing 6 (BIRC6), mRNA.
BIRC7	E21	NM_022161	Homo sapiens livin inhibitor-of-apotosis (LIVIN), mRNA.
BNIP-1	E24	NM_001205	Human BCL2/adenovirus E1B 19kD-interacting protein 1 (BNIP1)
BNIP-2	F24	NM_004330	Human BCL2/adenovirus E1B 19kD-interacting protein 2 (BNIP2)

Gene	Coord.	Genbank #	Gene name
BNIP-3	G24	NM_004052	Human BCL2/adenovirus E1B 19kD-interacting protein 3 (BNIP3)
BOK	H24	AF089746	Homo sapiens Bcl-2 related ovarian killer (BOK) mRNA, complete cds.
Bub1	A6	AF053305	Human mitotic checkpoint kinase Bub1 (BUB1) gene
Bub3	B6	AF081496	Homo sapiens kinetochore protein BUB3 (BUB3) mRNA
c-abl	F1	NM_005157	Homo sapiens v-abl Abelson murine leukemia viral oncogene homolog 1 (Abl-1)
c-fos	D8	K00650	Human Proto-oncogene
c-fyn	G1	NM_002037	Human FYN oncogene related to SRC, FGR, YES (FYN) gene
c-jun	E8	J04111	Human c-jun proto oncogene
c-lck	H1	NM_005356	Homo sapiens lymphocyte-specific protein tyrosine kinase (LCK)
c-lyn	I1	M16038	Human lyn mRNA encoding a tyrosine kinase
c-mpl	A11	M90102	Human (clones 15, 39, 41) c-myeloproliferative leukemia virus type P (c-mpl-P) mRNA
c-myb	F8	M15024	Human Proto-oncogene
c-myc	G8	E01841	Human proto-oncogene
c-rel	H8	NM_002908	Homo sapiens v-rel avian reticuloendotheliosis viral oncogene homolog (REL) mRNA.
c-src	J1	X59932	Human C-SRC-kinase
CAD	F21	AB013918	Homo sapiens mRNA for CAD
Calcineurin A1	K1	M29550	Human calcineurin A1 mRNA
Calcineurin B	L1	M30773	Human calcineurin B mRNA
CAMK2B	M1	NM_001220	Homo sapiens calcium/calmodulin-dependent protein kinase (CaM kinase) II beta (CAMK2B), mRNA.
CAMK2D	N1	NM_001221	Homo sapiens calcium/calmodulin-dependent protein kinase (CaM kinase) II delta (CAMK2D), mRNA.
Caspase 1	G21	NM_001223	Human apoptosis-related cysteine protease
Caspase 10	H21	NM_001230	Human apoptosis-related cysteine protease
Caspase 13	A22	AF078533	Homo sapiens evolutionarily related interleukin-1beta converting enzyme mRNA, complete cds.
Caspase 2	B22	NM_001224	Human apoptosis-related cysteine protease
Caspase 3	C22	NM_004346	Human apoptosis-related cysteine protease
Caspase 4	D22	NM_001225	Human apoptosis-related cysteine protease
Caspase 5	E22	NM_004347	Human apoptosis-related cysteine protease
Caspase 6	F22	NM_001226	Human apoptosis-related cysteine protease
Caspase 7	G22	NM_001227	Human apoptosis-related cysteine protease
Caspase 8	H22	NM_001228	Human apoptosis-related cysteine protease
Caspase 9	I22	NM_001229	Human apoptosis-related cysteine protease
CBL (c-cbl)	C19	X57110	Human c-cbl proto-oncogene
CBL-B	D19	U26710	Human cbl-b mRNA
CD27	E17	M63928	Homo sapiens T cell activation antigen (CD27) mRNA, complete cds.
CD27 Ligand	F17	L08096	Human CD27 ligand mRNA, complete cds.
CD30	G17	M83554	H.sapiens lymphocyte activation antigen CD30 mRNA, complete cds.
CD30 Ligand	A18	L09753	Homo sapiens CD30 ligand mRNA, complete cds.
CD4	B11	NM_000616	Homo sapiens CD4 antigen (p55) (CD4), mRNA.
CD40	B18	XM_009624	Homo sapiens tumor necrosis factor receptor superfamily, member 5 (TNFRSF5), mRNA

Gene	Coord.	Genbank #	Gene name
CD40 Ligand	C18	L07414	Human CD40-ligand mRNA, complete cds.
CD45	O1	Y00062	Human mRNA for T200 leukocyte common antigen (CD45, LC-A)
CDC2	C6	X05360	Human CDC2 gene involved in cell cycle control
Cdk2	D6	NM_001798	Human cyclin-dependent kinase 2 (CDK2)
Cdk3	E6	NM_001258	Human cyclin-dependent kinase 3 (CDK3)
Cdk4	F6	NM_000075	Human cyclin-dependent kinase 4 (CDK4)
Cdk5	G6	NM_004935	Human cyclin-dependent kinase 5 (CDK5)
Cdk6	H6	NM_001259	Human cyclin-dependent kinase 6 (CDK6)
Cdk7	I6	NM_001799	Homo sapiens cyclin-dependent kinase 7 (homolog of Xenopus MO15 cdk-activating kinase) (CDK7) mRNA.
Cdk8	J6	NM_001260	Homo sapiens cyclin-dependent kinase 8 (CDK8) mRNA.
CHEK1 (CHK1)	P1	NM_001274	Homo sapiens CHK1 (checkpoint, S.pombe) homolog (CHEK1), mRNA.
CHES1	K6	U68723	Human checkpoint suppressor 1 mRNA
CNTF	C11	X60542	Human CNTF gene for ciliary neurotrophic factor.
CNTFR	D11	NM_001842	Homo sapiens ciliary neurotrophic factor receptor (CNTFR), mRNA.
CRADD (RAIDD)	E19	NM_003805	Homo sapiens CASP2 and RIPK1 domain containing adaptor with death domain (CRADD), mRNA.
CREB1	I8	NM_004379	Homo sapiens cAMP responsive element binding protein 1 (CREB1)
CREB2	J8	M86842	Human cAMP response element regulatory protein (CREB2) gene
CrmA	J22	M14217	Cowpox virus white-pock variant (CPV-W2) (crmA) gene, complete cds.
CRYAB	F19	NM_001885	Homo sapiens crystallin, alpha B (CRYAB), mRNA.
CSF-1	E11	M76453	Human colony stimulating factor 1 (CSF1) gene
CSNK1A1 (CK1)	A2	NM_001892	Homo sapiens casein kinase 1, alpha 1 (CSNK1A1), mRNA.
Cyclin A1	L6	NM_003914	Homo sapiens cyclin A1 (CCNA1) mRNA.
Cyclin B1	A7	M25753	Human cyclin B mRNA, 3' end
Cyclin C	B7	NM_005190	Human cyclin C (CCNC) gene
Cyclin D1	C7	M64349	Human cyclin D1
Cyclin E	D7	M73812	Human cyclin E mRNA sequence.
Cyclin F	E7	NM_001761	Homo sapiens cyclin F (CCNF) mRNA.
Cyclin G1	F7	U53328	Human cyclin G
Cyto C1	G19	NM_001916	Homo sapiens cytochrome c-1 (CYC1) mRNA
DAP Kinase	B2	X76104	H.sapiens DAP-kinase mRNA
DAXX	H19	NM_001350	Homo sapiens death-associated protein 6 (DAXX), mRNA.
DDX1	I19	NM_004939	Homo sapiens DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 1 (DDX1)
DFFA	K22	NM_004401	Homo sapiens DNA fragmentation factor, 45 kD, alpha polypeptide (DFFA), mRNA.
DFFB	L22	NM_004402	Homo sapiens DNA fragmentation factor, 40 kD, beta polypeptide (caspase-activated DNase) (DFFB), mRNA.
DNA PK	C2	U47077	Homo sapiens DNA-dependent protein kinase catalytic subunit (DNA-PKcs) mRNA, complete cds.
DR6	D18	AF068868	Homo sapiens TNFR-related death receptor-6 (DR6) mRNA, complete cds.
DRAK1	D2	AB011420	Homo sapiens mRNA for DRAK1, complete cds.
DRAK2	E2	AB011421	Homo sapiens mRNA for DRAK2, complete cds.
E1Ac	K8	M18288	Adenovirus type 40 E1A and E1B genes
E2F-1	L8	M96577	Human (E2F-1) pRB-binding protein gene
EGF	F11	NM_001963	Human epidermal growth factor (EGF)
EGFR	G11	NM_005228	Human epidermal growth factor receptor

Gene	Coord.	Genbank #	Gene name
EPO	H11	NM_000799	Human erythropoietin (EPO)
EPOR	I11	NM_000121	Human erythropoietin receptor (EPOR)
ERK (MAPK1)	F2	XM_009860	Homo sapiens mitogen-activated protein kinase 1 (MAPK1), mRNA.
Ets1	M8	J04101	Human erythroblastosis virus oncogene homolog 1 (ets-1) mRNA
Ets2	N8	NM_005239	Homo sapiens v-ets avian erythroblastosis virus E26 oncogene homolog 2 (ETS2) mRNA.
FADD	J19	NM_003824	Human Fas (TNFRSF6)-associated via death domain (FADD)
FAK	G2	L13616	Human focal adhesion kinase (FAK) mRNA
FAP	H2	NM_004460	Homo sapiens fibroblast activation protein, alpha (FAP), mRNA.
Fas	E18	M67454	Human Fas Antigen
Fas-ligand	F18	D38122	Human Fas ligand gene
FGF R	C12	M34185	Human fibroblast growth factor receptor (FGFr) transmem brane form
FGF-1	J11	E03692	cDNA encoding human acidic FGF
FGF-2	K11	M27968	Human basic fibroblast growth factor (FGF) gene
FGF-3	A12	NM_005247	Human fibroblast growth factor 3 (murine mammary tumor virus integration site (v-int-2) oncogene homolog) (FGF3) gene
FGF-4	B12	NM_002008	Human fibroblast growth factor 4 (heparin secretory transforming protein 1, Kaposi sarcoma oncogene) (FGF4) gene
FLT-1	D12	AF063657	Homo sapiens vascular endothelial growth factor receptor (FLT1)
Fodrina	K19	XM_005369	Homo sapiens spectrin, alpha, non-erythrocytic 1 (alpha-fodrin) (SPTAN1), mRNA.
Fodrinb	A20	NM_003128	Homo sapiens spectrin, beta, non-erythrocytic 1 (SPTBN1), mRNA
fos B	O8	NM_006732	Homo sapiens FBJ murine osteosarcoma viral oncogene homolog B (FOSB), mRNA.
FRP-1	I2	U49844	Human FRAP-related protein (FRP1) mRNA
GADD45	G7	M60974	Human growth arrest and DNA-damage-inducible protein (gadd45)
GCK	J2	NM_000162	Homo sapiens glucokinase (hexokinase 4, maturity onset diabetes of the young 2) (GCK), nuclear gene encoding mitochondrial protein, mRNA.
GCSF	E12	NM_000759	Human colony stimulating factor 3 (granulocyte) (CSF3)
GMCSF	F12	NM_000758	Human colony stimulating factor 2 (granulocyte-macrophage) (CSF2)
GPRK6	K2	XM_003736	Homo sapiens G protein-coupled receptor kinase 6 (GPRK6), mRNA.
GPX1	B20	M21304	Human glutathione peroxidase (GPX1) mRNA, complete cds.
Granzyme A	M22	NM_006144	Homo sapiens granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3) (GZMA), mRNA.
Granzyme B	N22	M28879	Human granzyme B (CTLA-1) gene
GSK3B	L2	NM_002093	Homo sapiens glycogen synthase kinase 3 beta (GSK3B), mRNA.
GSTM1	C20	NM_000561	Homo sapiens glutathione S-transferase M1 (GSTM1), mRNA
Hrk	I24	U76376	Homo sapiens activator of apoptosis Hrk (HRK) mRNA, complete cds.

Gene	Coord.	Genbank #	Gene name
HSF-1 (TCF5)	E20	M64673	Human heat shock factor 1 (TCF5) mRNA, complete cds.
HsRad51	A9	D13804	Human mRNA for RecA-like protein HsRad51, complete cds.
I-FLICE (FLIP)	L23	AF041462	Homo sapiens I-FLICE isoform 5 mRNA, complete cds.
ICSBP1	B9	NM_002163	Homo sapiens interferon consensus sequence binding protein 1 (ICSBP1), mRNA.
IFN-a	G12	NM_002171	Homo sapiens interferon, alpha 10 (IFNA10), mRNA.
IFN-b	J12	M35591	Human fibroblast interferon (IFN-beta) gene
IFN-g	K12	J00219	Human immune interferon (IFN-gamma) gene
IFNAR1	H12	NM_000629	Homo sapiens interferon (alpha, beta and omega) receptor 1 (IFNAR1), mRNA.
IFNAR2	I12	NM_000874	Homo sapiens interferon (alpha, beta and omega) receptor 2 (IFNAR2), mRNA.
IFNGR1	L12	NM_000416	Homo sapiens interferon gamma receptor 1 (IFNGR1), mRNA.
IFNGR2	M12	NM_005534	Homo sapiens interferon gamma receptor 2 (interferon gamma transducer 1) (IFNGR2), mRNA.
IGF-1	N12	M29644	Human insulin-like growth factor I gene
IGF-2	O12	M29645	Human insulin-like growth factor II gene
IGFBP1	P12	M31145	Human insulin-like growth factor binding protein mRNA, complete cds.
IK	A13	NM_006083	Homo sapiens IK cytokine, down-regulator of HLA II (IK), mRNA.
IkB (MAD3)	C9	M69043	Human MAD-3 mRNA encoding IkB-like activity
IKK-a	N2	AF080157	Homo sapiens IkB kinase-a (IKK-alpha) mRNA
IKK-b	O2	AF080158	Homo sapiens IkB kinase-b (IKK-beta) mRNA
IKK-g	P2	AF074382	Homo sapiens IkB kinase gamma subunit (IKK-gamma) mRNA
IL-10	B13	U16720	Human interleukin 10 (IL10) gene
IL-10Ra	C13	NM_001558	Homo sapiens interleukin 10 receptor, alpha (IL10RA), mRNA.
IL-10Rb	D13	NM_000628	Homo sapiens interleukin 10 receptor, beta (IL10RB), mRNA.
IL-11	E13	M57765	Human interleukin 11 mRNA
IL-11RA	F13	NM_004512	Homo sapiens interleukin 11 receptor, alpha (IL11RA), mRNA.
IL-12 p35	G13	AF180562	Homo sapiens interleukin 12, P35 mRNA
IL-12 p40	H13	AF180563	Homo sapiens interleukin 12, P40 gene
IL-12RB1	I13	NM_005535	Homo sapiens interleukin 12 receptor, beta 1 (IL12RB1), mRNA.
IL-12RB2	J13	NM_001559	Homo sapiens interleukin 12 receptor, beta 2 (IL12RB2), mRNA.
IL-13	K13	L06801	Homo sapiens interleukin 13 mRNA, complete cds.
IL-13RA1	L13	NM_001560	Homo sapiens interleukin 13 receptor, alpha 1 (IL13RA1), mRNA.
IL-13RA2	M13	NM_000640	Homo sapiens interleukin 13 receptor, alpha 2 (IL13RA2), mRNA.
IL-14	N13	L15344	Human high molecular weight B cell growth factor mRNA sequence.
IL-15	O13	U14407	Human interleukin 15 (IL15) mRNA, complete cds.
IL-15RA	P13	NM_002189	Homo sapiens interleukin 15 receptor, alpha (IL15RA), mRNA.
IL-16	A14	NM_004513	Homo sapiens interleukin 16 (lymphocyte chemoattractant factor) (IL16), mRNA.

Gene	Coord.	Genbank #	Gene name
IL-17	B14	U32659	Human IL-17 mRNA, complete cds.
IL-17B	C14	NM_014443	Homo sapiens interleukin 17B (IL17B), mRNA.
IL-17BR	D14	AF212365	Homo sapiens IL-17B receptor (IL17BR) mRNA, complete cds.
IL-17R	E14	NM_014339	Homo sapiens interleukin 17 receptor (IL17R), mRNA.
IL-18	F14	NM_001562	Homo sapiens interleukin 18 (interferon-gamma-inducing factor)(IL18), mRNA.
IL-18BP	G14	NM_005699	Homo sapiens interleukin 18 binding protein (IL18BP), mRNA.
IL-18R1	H14	NM_003855	Homo sapiens interleukin 18 receptor 1 (IL18R1), mRNA.
IL-19	I14	XM_001404	Homo sapiens interleukin 19 (IL19), mRNA.
IL-1a	J14	X03833	Human gene for interleukin 1 alpha (IL-1 alpha)
IL-1b	K14	M15330	Human interleukin 1-beta (IL1B) mRNA, complete cds.
IL-1R1	L14	NM_000877	Homo sapiens interleukin 1 receptor, type I (IL1R1), mRNA.
IL-1R2	M14	NM_004633	Homo sapiens interleukin 1 receptor, type II (IL1R2) mRNA
IL-1RL1	N14	NM_016232	Homo sapiens interleukin 1 receptor-like 1 (IL1RL1), mRNA.
IL-1RL2	O14	NM_003854	Homo sapiens interleukin 1 receptor-like 2 (IL1RL2), mRNA.
IL-2	P14	U25676	Human interleukin 2
IL-20	A15	NM_018724	Homo sapiens interleukin 20 (IL20), mRNA.
IL-21	B15	AF254069	Homo sapiens interleukin 21 (IL21) mRNA, complete cds.
IL-21R	C15	AF254067	Homo sapiens interleukin 21 receptor (IL21R) mRNA, complete cds.
IL-22	D15	NM_020525	Homo sapiens interleukin 22 (IL22), mRNA.
IL-22R	E15	AF286095	Homo sapiens IL-22 receptor (IL22R) mRNA, complete cds.
IL-2R b	G15	NM_000878	Homo sapiens interleukin 2 receptor, beta (IL2RB), mRNA.
IL-2R g	H15	NM_000206	Homo sapiens interleukin 2 receptor, gamma (severe combined immunodeficiency) (IL2RG), mRNA.
IL-2Ra	F15	NM_000417	Homo sapiens interleukin 2 receptor, alpha (IL2RA), mRNA.
IL-3	I15	M14743	Human interleukin 3 (IL-3)
IL-3Ra	J15	M74782	Human interleukin 3 receptor (hIL-3Ra) mRNA
IL-4	K15	NM_000589	Human interleukin 4 (IL4)
IL-4Ra	L15	NM_000418	Homo sapiens interleukin 4 receptor (IL4R), mRNA.
IL-5	M15	J03478	Human interleukin 5 (IL-5) gene
IL-5Ra	N15	M96652	Human interleukin 5 receptor alpha-subunit (IL5R) mRNA, complete cds.
IL-6	O15	M54894	Human interleukin 6 mRNA
IL-6R	A16	X12830	Human mRNA for interleukin-6 (IL-6) receptor
IL-6ST	B16	NM_002184	Homo sapiens interleukin 6 signal transducer (gp130, oncostatin M receptor) (IL6ST), mRNA.
IL-7	C16	J04156	Human interleukin 7 (IL-7)
IL-7R	D16	M29696	Human interleukin-7 receptor (IL-7) mRNA
IL-8	E16	M28130	Human interleukin 8 (IL8) gene
IL-8R	F16	M68932	Human IL-8 receptor mRNA
IL-8Rb	G16	NM_001557	Homo sapiens interleukin 8 receptor, beta (IL8RB), mRNA.
IL-9	H16	XM_003760	Homo sapiens interleukin 9 (IL9), mRNA.
IL-9R	I16	NM_002186	Homo sapiens interleukin 9 receptor (IL9R), mRNA.
iNOS	F20	L09210	Homo sapiens inducible nitric oxide synthase mRNA, complete cds.
INPP5D (SHIP)	A3	NM_005541	Homo sapiens inositol polyphosphate-5-phosphatase, 145kD (INPP5D), mRNA.
Insulin	J16	J00265	Human insulin gene
Insulin Receptor	K16	XM_048346	Homo sapiens insulin receptor (INSR), mRNA.
IRAP (IL-1RP)	L16	X53296	H.sapiens mRNA for IRAP.
IRF1	D9	NM_002198	Homo sapiens interferon regulatory factor 1 (IRF1) mRNA

Gene	Coord.	Genbank #	Gene name
IRF2	E9	NM_002199	Homo sapiens interferon regulatory factor 2 (IRF2), mRNA.
IRF4	F9	U52682	Human lymphocyte specific interferon regulatory factor/ interferon regulatory factor 4 (LSIRF/IRF4) mRNA
JAK1	B3	M64174	Human protein-tyrosine kinase (JAK1) gene
JAK2	C3	NM_004972	Human Janus kinase 2 (a protein tyrosine kinase) (JAK2) gene
JAK3	D3	U09607	Human JAK family protein tyrosine kinase (JAK3) gene
JNK2	E3	NM_002752	Homo sapiens mitogen-activated protein kinase 9 (MAPK9), mRNA.
JNK3A1	F3	U34820	Human JNK3 alpha1 protein kinase (JNK3A1) mRNA, complete cds.
JNK3A2	G3	U34819	Human JNK3 alpha2 protein kinase (JNK3A2) mRNA, complete cds.
JunB	G9	X51345	Human jun-B mRNA for JUN-B protein.
JunD	H9	X51346	Human jun-D mRNA for JUN-D protein
KIT	M16	NM_000222	Homo sapiens v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT), mRNA.
L-myc	I9	M19720	Human L-myc protein gene
Lamin A	J9	NM_005572	Homo sapiens lamin A/C (LMNA), mRNA.
Lamin B1	K9	NM_005573	Homo sapiens lamin B1 (LMNB1), mRNA.
Large T Ag	L9	J02400	Simian virus 40 Large T Antigen
mad	M9	L06895	Human antagonizer of myc transcriptional activity
MADD	G20	NM_003682	Homo sapiens MAP-kinase activating death domain (MADD) mRNA
MAP4	H20	U19727	Human microtubule-associated protein 4 (MAP4) mRNA, complete cds.
MAPKAPK2	H3	NM_004759	Homo sapiens mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2), transcript variant 1, mRNA.
MAPKAPK5	I3	XM_027021	Homo sapiens mitogen-activated protein kinase-activated protein kinase 5 (MAPKAPK5), mRNA.
max	N9	X60287	Human max gene
MCL1	J24	NM_021960	Homo sapiens myeloid cell leukemia sequence 1 (BCL2-related) (MCL1), mRNA.
MDM2	I20	Z12020	Human p53-associated gene
MEK1	J3	L11284	Human ERK activator kinase (MEK1) gene
MEK2	K3	L11285	Homosapiens ERK activator kinase (MEK2) mRNA
MEK3	L3	NM_002756	Homo sapiens mitogen-activated protein kinase kinase 3 (MAP2K3),
MEK5	M3	NM_002757	Homo sapiens mitogen-activated protein kinase kinase 5 (MAP2K5),
MEK6	N3	U39657	Human MAP kinase kinase 6 (MKK6) mRNA
MEKK1	O3	AAC97073	MEK kinase 1
MEKK2	P3	NM_006609	Homo sapiens mitogen-activated protein kinase kinase kinase 2 (MAP3K2), Mrna
MEKK3	A4	U78876	Human MEK kinase 3 gene
MEKK4	B4	NM_005922	Homo sapiens mitogen-activated protein kinase kinase kinase 4 (MAP3K4), transcript variant 1, mRNA
MEKK5 (ASK1)	C4	NM_005923	Homo sapiens mitogen-activated protein kinase kinase kinase 5 (MAP3K5) mRNA
MKK4	D4	U17743	Human JNK activating kinase (JNKK1) mRNA
MKP-2 (DUSP4)	E4	U48807	Human MAP kinase phosphatase (MKP-2) mRNA, complete cds.
MLK	F4	AF238255	Homo sapiens mixed lineage kinase mRNA, complete cds.
MMP1	M23	U41078	Human membrane-type matrix metalloproteinase-1 mRNA
MNK2	G4	AF125532	Homo sapiens map kinase-interacting kinase mRNA, complete cds.

Gene	Coord.	Genbank #	Gene name
Myt1	H4	U56816	Human kinase Myt1 (Myt1) mRNA, complete cds.
n-myc	O9	Y00664	Human germ line n-myc gene
NAIP (BIRC1)	N23	U19251	Homo sapiens neuronal apoptosis inhibitory protein mRNA, complete cds.
NFAT-1b	P9	U43341	Human transcription factor NFAT1 isoform B (NFAT1) mRNA
NFAT4	A10	L41067	Homo sapiens NF-AT4c mRNA
NFAT5	B10	AF134870	Homo sapiens transcription factor NFAT5 mRNA
NFkB1	C10	NM_003998	Homo sapiens nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105) (NFKB1), mRNA.
NFkB2	D10	X61498	H.sapiens mRNA for NF-kB subunit
NGF-2	G18	X53655	H.sapiens mRNA for NGF-2
NGF-b	H18	NM_002506	Human nerve growth factor, beta polypeptide (NGFB) gene
NGFR	L18	M14764	Human nerve growth factor receptor mRNA
NIK	I4	Y10256	H.sapiens mRNA for serine/threonine protein kinase
NuMa	E10	Z11583	H.sapiens mRNA for NuMA protein.
OPG	I18	U94332	Human osteoprotegerin (OPG) mRNA, complete cds.
OX40	J18	X75962	H.sapiens mRNA for OX40 homologue.
OX40 Ligand	K18	X79929	H.sapiens OX40 ligand/gp34.
p15	H7	L36844	Human (clone p15INK4B/HA5) CDK inhibitory protein gene
p16	I7	L27211	Human CDK4-inhibitor (p16-INK4) gene
p18	J7	U17074	Human CDK6 inhibitor p18 gene
p19	K7	U40343	Human CDK inhibitor p19INK4d gene
p27	L7	X84849	Human p27kip1 gene
p53	F10	K03199	Human p53 Tumor Antigen
p57	M7	U48869	Human cdk-inhibitor p57/KIP2 (CDKN1C) gene
p70 S6K (SRK)	J4	AB019245	Human p70 S6Kb S6 kinase b gene
PA2G4	N7	NM_006191	Homo sapiens proliferation-associated 2G4, 38kD (PA2G4) mRNA
PAK1	K4	NM_002576	Homo sapiens p21/Cdc42/Rac1-activated kinase 1 (yeast Ste20-related) (PAK1) mRNA
PARP	G10	NM_001618	Human ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase) (ADPRT) gene
PCNA	O7	NM_002592	Human proliferating cell nuclear antigen (PCNA) gene
PDGF R	H17	J03278	Human platelet-derived growth factor (PDGF) receptor mRNA
PDGF-a	N16	X06374	Human mRNA for platelet-derived growth factor PDGF-A
PDGF-b	O16	X02811	Human platelet-derived growth factor B chain (PDGF-B) gene
perforin	O23	M28393	Human perforin mRNA, complete cds.
PFTK1	L4	NM_012395	Homo sapiens PFTAIRE protein kinase 1 (PFTK1), mRNA.
PI3 Kinase	M4	Y11312	Human gene for phosphoinositide 3-kinase
Pitslare A1	N4	U04815	Human protein kinase PITSLRE alpha 1 mRNA
PKA-a	O4	X07767	Human mRNA for cAMP-dependent protein kinase catalytic subunit type alpha
PKC-a	P4	X52479	Human PKC alpha protein kinase C alpha gene
PKC-b1	A5	X06318	Human gene protein kinase C (PKC) type beta I
PKC-delta	B5	NM_006254	Homo sapiens protein kinase C, delta (PRKCD) mRNA
PKC-r	C5	Z15114	H.sapiens gene for protein kinase C gamma
PLK	D5	NM_005030	Homo sapiens polo (Drosophia)-like kinase (PLK), mRNA.
PP2A cs	F5	XM_003944	Homo sapiens protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform (PPP2CA), mRNA.
PPP1CB	E5	NM_002709	Homo sapiens protein phosphatase 1, catalytic subunit, beta isoform
PPP2R5A	G5	XM_010692	Homo sapiens protein phosphatase 2, regulatory subunit B (B56), alpha isoform (PPP2R5A), mRNA.

Gene	Coord.	Genbank#	Gene name
presenilin 1	J20	L76517	Homo sapiens (clone cc44) senilin 1 (PS1; S182) mRNA, complete cds.
PU.1 (SPI1)	H10	X52056	Human mRNA for spi-1 proto-oncogene
PYK2 (FAK2)	H5	U33284	Human protein tyrosine kinase PYK2 mRNA, complete cds.
Raf (A-Raf-1)	A1	X04790	Human mRNA for A-raf-1 oncogene
Raf (c-raf1)	I5	X03484	Human mRNA for raf oncogene
RAN	I10	NM_006325	RAN, member RAS oncogene familyHomo sapiens RAN, member RAS oncogene family (RAN), mRNA
RANKL (TRANCE)		M18	AF019047
Homo sapiens receptor activator of nuclear factor kappa B ligand (RANKL) mRNA, complete cds.			
rasGAP	K20	D78155	Homo sapiens mRNA for rasGTPase activating protein, complete cds.
Rb (p110)	J10	M28419	Human retinoblastoma susceptibility protein mRNA
Rb2 (p130)	K10	X74594	Human Rb2/p130 protein
RelA	L10	L19067	Human NF-kappa-B transcription factor p65 subunit mRNA
RelB	M10	NM_006509	Human v-rel avian reticuloendotheliosis viral oncogene homolog B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 3) (RELB) gene
RIP3	J5	AF156884	Homo sapiens RIP-like kinase (RIP3) mRNA, complete cds.
RIPK1	K5	NM_003804	Homo sapiens receptor (TNFRSF)-interacting serine-threonine kinase 1 (RIPK1) mRNA
RIPK2	L5	NM_003821	Homo sapiens receptor-interacting serine-threonine kinase 2 (RIPK2), mRNA.
RPS6KA6	M5	AF184965	Homo sapiens ribosomal S6 kinase (RPS6KA6) mRNA, complete cds.
SAPK4	N5	AF004709	Homo sapiens stress-activated protein kinase 4 mRNA, complete cds.
SCF	I17	M59964	Human stem cell factor mRNA
Sentrin	L20	U83117	Human sentrin mRNA, complete cds.
SOD1	M20	X02317	Human mRNA for Cu/Zn superoxide dismutase (SOD).
SPHK1	O5	NM_021972	Homo sapiens sphingosine kinase 1 (SPHK1), mRNA.
SPHK2	P5	AF245447	Homo sapiens sphingosine kinase type 2 isoform mRNA, complete cds.
Stat1	N10	M97935	Homo sapiens transcription factor ISGF-3 mRNA
Stat2	O10	NM_005419	Homo sapiens signal transducer and activator of transcription 2, 113kD (STAT2) mRNA
Stat3	P10	AJ012463	Homo sapiens mRNA for transcription factor
Stat4	L11	L78440	Human STAT4 gene
Stat5a	M11	U43185	Human signal transducer and activator of transcription Stat5A gene
Stat6	N11	NM_003153	Human signal transducer and activator of transcription 6, interleukin-4 induced (STAT6) gene
TAB1	N20	U49928	Homo sapiens TAK1 binding protein (TAB1) mRNA, complete cds.
TAK1 (MAP3K7)	M6	AF218074	Homo sapiens TGF beta-activated kinase splice variant d (TAK1) mRNA, complete cds.
TANK (TRAF2)	O20	U59863	Human TRAF-interacting protein I-TRAF mRNA, complete cds.
TGF-a	J17	M31172	Human transforming growth factor-alpha gene
TGF-b	K17	X02812	Human transforming growth factor-beta gene
TGFb-RI	L17	NM_004612	Homo sapiens transforming growth factor, beta receptor I (activin A receptor type II-like kinase, 53kD) (TGFBR1) mRNA
TGFb-RII	M17	NM_003242	Homo sapiens transforming growth factor, beta receptor II (70-80kD) (TGFBR2) mRNA

Gene	Coord.	Genbank #	Gene name
TGFb-RIII	N17	NM_003243	Homo sapiens transforming growth factor, beta receptor III (betaglycan, 300kD) (TGFBR3) mRNA
TIE-1	N6	AF023676	Homo sapiens lamin B receptor homolog TM7SF2 (TM7SF2) mRNA
TIE-2 (TEK)	O6	L06139	Homo sapiens receptor protein-tyrosine kinase (TEK) mRNA, complete cds.
TIMP-1	P23	NM_003254	Homo sapiens tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor) (TIMP1) mRNA.
TNF	N18	X01394	Human tumor necrosis factor
TNF-R1	O18	X55313	Human TNF-R gene for tumor necrosis factor receptor type 1
TNF-R2	P18	U52156	Human tumor necrosis factor receptor 2 (TNFR2) gene
TNFAIP3	P20	NM_006290	Homo sapiens tumor necrosis factor, alpha-induced protein 3 (TNFAIP3), mRNA.
TPO	O17	NM_000460	Human thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development factor) (THPO) gene
TR3 (nur77)	O11	L13740	Human TR3 orphan receptor mRNA, complete cds.
TRADD	I21	L41690	Homo sapiens TNF receptor-1 associated protein (TRADD) mRNA
TRAF1	J21	NM_005658	Homo sapiens TNF receptor-associated factor 1 (TRAF1) mRNA
TRAF3 (CRAF1)	K21	NM_003300	Homo sapiens TNF receptor-associated factor 3 (TRAF3), mRNA.
TRAF4	L21	NM_004295	Homo sapiens TNF receptor-associated factor 4 (TRAF4) mRNA
TRAF5	M21	AB000509	Homo sapiens mRNA for TRAF5, complete cds.
TRAF6	N21	NM_004620	Homo sapiens TNF receptor-associated factor 6 (TRAF6) mRNA
TRAIL	P19	U37518	Human TNF-related apoptosis inducing ligand TRAIL gene
TRAIL-R1 (DR4)	L19	U90875	Human cytotoxic ligand TRAIL receptor mRNA, complete cds.
TRAIL-R2 (DR5)	M19	AF012535	Homo sapiens death receptor 5 (DR5) mRNA, complete cds.
TRAIL-R3 (DCR1)	N19	AF020502	Homo sapiens cytotoxic TRAIL receptor-3 (TRAIL-R3) mRNA, complete cds.
TRAIL-R4 (DCR2)	O19	AF029761	Homo sapiens decoy receptor 2 mRNA, complete cds.
TRIP	O21	NM_005879	Homo sapiens TRAF interacting protein (TRIP), mRNA.
TRPM-2	P21	M64722	Human TRPM-2 mRNA, complete cds.
VEGF	P17	E14233	Human mRNA for vascular endothelial growth factor (VEGF) gene
WAF1	P11	U03106	Human wild-type p53 activated fragment-1 (WAF1) mRNA, complete cds.
Wee 1	P6	NM_003390	Human wee1+ (S. pombe) homolog (WEE1) mRNA

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Biotechnologies, Inc.

Profiler™ Array Nonradioactive System User Manual

(Version 2.2)

STORAGE
Store Box 1 at room temperature (RT), Box 2 at –20°C, and Box 3 at 4°C. Please note that the Substrate is light sensitive and should be stored in the dark.

FOR RESEARCH USE ONLY.

TABLE OF CONTENTS

I. INTRODUCTION

With the advent of macroarray technology, studying differential gene expression has become relatively easy and routine. The expression profiles of hundreds of genes can be studied simultaneously in a single array experiment. Typically, array experiments require the use of radioactive cDNA probes. However, working with radioactively labeled probes is a hassle, requiring time-consuming procedures. Furthermore, radioactively labeled cDNA arrays tend to generate high levels of background.

The Profiler™ Array provides researchers with an alternative approach—a hassle-free, nonradioactive array system that employs digoxigenin (DIG)-11-dUTP-labeled cDNA and chemiluminescent detection. With this method, you can obtain semiquantitative results in just 1–2 days, compared to 4–7 days that radioactive-based methods demand.

Our first array, the Star Profiler Array, contains 373 genes—an exclusive set of the most popular and well-studied genes—spotted onto a positively charged nylon membrane. Star Profiler Array contains genes that play a significant role in biological functions and are corroborated with published literature; thus, a single experiment using Star Profiler Arrays generates highly informative data. In addition, each spot contains long oligonucleotides (≥90 nt), synthesized from a carefully selected sequence. Our selection process eliminates any homologous regions among the genes on the array so there is no risk of crosshybridization. As a result, your labeled cDNA probe will hybridize efficiently and selectively to specific sequences on the array. In addition, we offer targeted arrays such as the Oncogene Profiler Array and Apoptosis Profiler Array. For a complete list of genes for each array, see the Product Certificate Sheet provided with each kit.

Benefits of the Profiler™ Array

Unlike other macroarrays, which often require poly-A^+ RNA, the Profiler Array has no preference for total or poly-A^+ RNA. In fact, our method provides equivalent results using either total RNA or mRNA, so there is no need for special RNA purification procedures.

I. INTRODUCTION *continued*

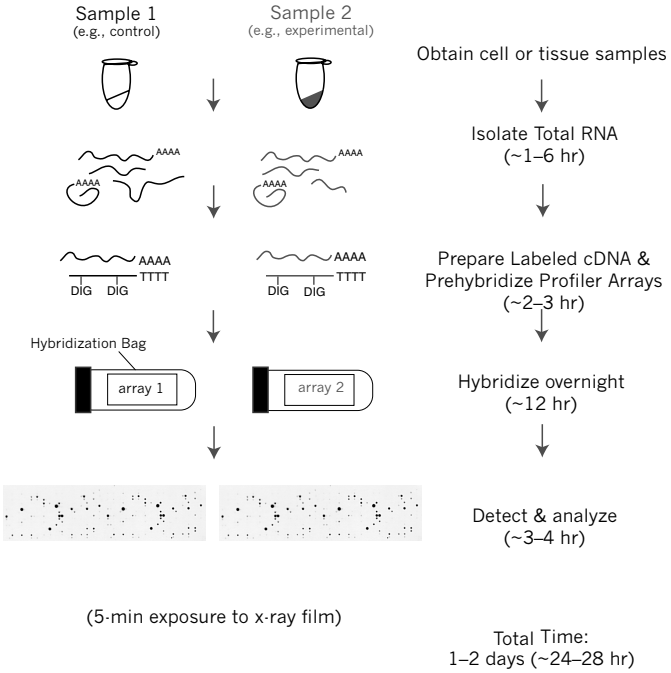


Figure 1 provides a simplified overview of the Profiler procedure. After cDNA labeling, Profiler Arrays are hybridized overnight with DIG-11-dUTP-labeled cDNA. The next day, the array membranes are washed and subsequently incubated with Anti-DIG-Alkaline Phosphatase (DIG-AP) Antibody. Upon addition of the CDP-Star chemiluminescent substrate (provided), detection of DIG-AP can be obtained within minutes by exposing array membranes to x-ray film or by using a chemiluminescent-imaging system, such as Alpha Innotech's FluorChem Imaging System.

II. PACKAGE CONTENTS

See the front cover of this User Manual for appropriate storage conditions. For Buffer Solutions & Recipes, see Appendix at the back of this User Manual.

Profiler Array Nonradioactive System†

Profiler Array and Hybridization Kit (Store at room temp.)
- 2 Profiler Arrays
- 25 ml Hybridization Buffer
- 60 ml 20X SSC
- 60 ml 10% SDS

Profiler Array cDNA Synthesis Kit (Store at –20°C)
- 10 µg Control K562 Total RNA (yellow)
- 10 µl Reverse Transcriptase (amber)
- 15 µl Random Primer Mix (blue)
- 15 µl Probe Mix (pink)
- 50 µl 100mM DTT (salmon)
- 100 µl 5X RT Buffer (purple)
- 100 µl dH_20 (natural)

Profiler Array Chemiluminescence Detection Kit (Store at 4°C)
- 15 ml CDP-*Star* Substrate
- 30 ml 5X Blocking Buffer
- 50 ml 10X Antibody Wash Buffer I
- 50 ml 10X Antibody Wash Buffer II
- 30 ml 10X Detection Buffer
- 15 µl Alk-Phosphatase Conjugate (orange)

Additional Materials Provided
- 1 Profiler Grid
- 1 Product User Manual

†See Product Analysis Certificate provided with each kit for complete list of genes spotted on the array.

III. ADDITIONAL MATERIALS & EQUIPMENT

Additional Materials Required

- Sheared salmon testes DNA (Sigma, Cat. #D7656)
- Hyperfilm ECL Film (Amersham, Cat. #RPN31144)
- Plastic wrap (e.g., Saran Wrap) or plastic sheet protectors
- Commercial pouch sealer or equivalent

Additional Materials Recommended

- Trizol (Life Technologies, Cat. #15596-018) or equivalent

Additional Equipment

- Thermal cycler or water bath
- Temperature controlled Shaking or Rotating platform
- [Optional] Hybridization oven and bottles (Stratagene Cat. #401031)
- [Optional] Alpha Innotech FluorChem or equivalent

IV. INSTRUCTIONS FOR USE

A. Isolation of total RNA from cells or tissues

Total RNA can be isolated from tissues or cells using standard methods (e.g., Trizol or equivalent). You can use poly-A^+ RNA if you wish; however, we have found that equivalent results can be achieved using total RNA.

Quantify your RNA samples by measuring the absorbance at 260 nm and 280 nm. Total RNA samples that are free of proteins have an A_{260}/A_{280} ratio of ~1.8–2.2. In addition, you should check the quality of your RNA by agarose gel electrophoresis. Run 0.5–2.0 μg of RNA on a denaturing formaldehyde/agarose/ethidium-bromide gel. Two bright bands, 28S and 18S rRNA, should be observed at approximately 5 and 2 kb, respectively. These bands are sharply defined and visible at about a 2:1 ratio (28S:18S).

B. Preparation of cDNA probe

For best results, we recommend using a thermal cycler for all incubation steps; however, if a thermal cycler is unavailable, heating blocks may be used. If desired, first perform prehybridization (Step C.1–4), and then prepare RNA (Step B.2) and cDNA synthesis mixture (Step B.4), simultaneously.

1. Preheat a thermal cycler or heating block (or equivalent) to 70°C.

IV. INSTRUCTION FOR USE *continued*

2. For each total RNA sample, combine the following components into a sterile 0.5-ml microcentrifuge tube:

5–10 µg	Total RNA
3 µl	Random Primer Mix

   Bring each tube to a final volume of 10 µl by adding dH_2O (provided). Mix well by pipetting and spin briefly in a microcentrifuge. Keep on ice.
3. Heat samples at 72°C for 2 min. Then reduce temperature to 42°C and incubate for 1–2 min.

   Note: If a heating block is used, tubes must be incubated for a minimum of 2 min at 42°C.
4. Prepare the cDNA synthesis mixture for each total RNA sample:

6 µl	5X First Strand Buffer†
3 µl	0.1 M DTT†
6 µl	Probe Mix
1 µl	Reverse Transcriptase
4 µl	H_2O (provided)
20 µl	**Total volume per RNA sample**

5. Mix well by pipetting. Incubate the cDNA synthesis mixture at 42°C for 2 min.
6. Transfer the mixture to the tube containing the RNA sample (from Step B3) and mix well by pipetting. Then incubate at 42°C for 2 hr.
7. After incubation, samples at 75°C for 10 min to inactivate RT. Proceed directly to Step D (Hybridization) or store DIG-labeled cDNA probe(s) at –20°C until use.

C. Pre-Hybridization

Before you start, preheat Hybridization Buffer to 52°C. Completely dissolve all precipitates in the Hybridization Buffer. Prepare sheared salmon testes DNA:

1. Heat the sheared salmon testes DNA (~10 mg/ml) at 95–100°C for 5 min, then immediately place on ice (~5 min). We recommend preparing aliquots of 100 µl (i.e., 50 µl per membrane), then storing at –20°C until use.
2. Place each membrane into a hybridization pouch with the spots facing up. (Note the orientation of the membranes. The correct orientation is shown in the Appendix, indicating that the notch is on the top, right-hand corner.) Fill the pouch entirely with pre-warmed 5 ml deionized H_2O; avoid adding H_2O directly onto the membrane. Then carefully decant water.
4. For each membrane, prepare pre-hybridization solution: Add 50 µl of the sheared salmon testes DNA to 5ml of pre-warmed Hybridization Buffer. After mixing, add 5 ml of pre-hybridization solution to each pouch containing the membrane. Remove bubbles before sealing the pouch. Incubate at 52°C for ≥2 hr.

IV. INSTRUCTIONS FOR USE *continued*

D. Hybridization

1. Heat DIG-labeled cDNA probe at 94°C for 5 min. Then incubate on ice for 5 min.
2. Cut open hybridization pouch, add each DIG-labeled cDNA probe directly to the Hybridization Buffer (Step C.4), reseal pouch and hybridize overnight (≥12 hours) at 52°C.
3. The next day, prepare SSC/SDS washing solutions as described in the Appendix. Preheat SSC/SDS solutions to 52°C before use. Ensure that the SSC/SDS solutions are free from all precipitates. Shake well before use.
4. Cut open hybridization pouch, decant cDNA probe/buffer and place membrane into a clear plastic container such as a 200 µl pipet tip container. Quicky rinse each membrane with 10 ml 2X SSC/0.5% SDS and decant. Wash twice with 30 ml of prewarmed 2X SSC/0.5% SDS at 52°C for 10 min per wash. Then wash twice using 30 ml of prewarmed 0.1X SSC/0.5% SDS at 52°C for 10 min per wash. Keep SSC/SDS solutions at 52°C during wash steps.

E. Detection

Note that all incubation and wash steps, below, should be performed on a rotating platform with moderate shaking at room temp.

1. **Blocking**: Decant 0.1X SSC/0.5% SDS washing solution and add 30 ml of 1X Blocking Buffer to each container.
2. Incubate at room temperature for 30 min.
3. **Prepare anti-DIG-AP Antibody Mixture**: For each membrane, mix 1 ml of 1X Blocking Buffer and 6 µl of anti-DIG-AP Antibody. Mix well. Add the 1-ml mixture to each container, and incubate at room temperature for 30 min.

   Note: Be sure to spin the antibody conjugate for 5 minutes at maximum speed before diluting in 1X Blocking Buffer. Some lots may contain aggregated conjugates that precipitate and can cause a spotty background on the membrane.
4. **Wash the membranes at room temperature:** Remove the anti-DIG-AP Antibody Mixture/Blocking Buffer, and wash twice using 40 ml of 1X Antibody Wash Buffer I for 10 min per wash. Then repeat washes using 40 ml of 1X Antibody Wash Buffer II for 10 min per wash.
5. Dilute CDP-Star Substrate in 1X Detection Buffer (1:30 for x-ray film or 1:10 for Imager). Incubate each membrane in 30 ml diluted substrate for 30 minutes at room temperature.
6. Using forceps, remove each membrane from the diluted substrate. Do not allow the membrane to dry. We recommend placing the membrane on a plastic support, such as a developed/used x-ray film.

IV. INSTRUCTIONS FOR USE *continued*

7. Immediately cover each membrane with plastic wrap (e.g., Saran Wrap) or plastic sheet protectors, and remove excess substrate. Avoid air bubbles. Expose the membrane to Hyperfilm ECL (Amersham Cat. #RPN31144) for 3–10 min at room temperature. We suggest trying several different exposures of varying lengths of time (i.e., 2–10 min). Alternatively, use a chemiluminescent-imaging system, such as Alpha Innotech's FluorChem Imager or equivalent.

F. Interpretation of results

For quantitative results, we suggest using an chemiluminescent-imaging system such as Alpha Innotech's FluorChem or equivalent. Be sure to read Section G (below) to find out how to normalize the genes on the arrays. For a quick, cursory analysis of results, you may perform the following:

1. Place the two x-ray images together such that the housekeeping genes are aligned.
2. Slowly shift one x-ray film to the left or right, and observe any differences in spot intensity. Spots that exhibit differences in signal intensity may indicate that a gene is upregulated or downregulated; however, results are not conclusive until you have normalized the genes on the arrays.

G. Normalization

On each array, two housekeeping genes (beta-actin and ubiquitin), have been spotted at different locations on the array. Four beta-actin spots have been placed on the lower right hand corner for orientation purposes, see Appendix. Select one housekeeping gene. On each array, take the average intensity for the housekeeping gene of your choice and use this value as the baseline. Normalize each spot with respect to the average value of the housekeeping gene. After normalizing each array, compare spot intensities on separate arrays for each gene. Generally, a threefold increase or decrease in spot intensity is considered significant.

V. TROUBLESHOOTING GUIDE

The following list of tips is designed to help you troubleshoot problems that you may encounter when performing the procedure. If you feel you need additional technical assistance, please contact us by phone, fax, or email.

A. Problem: high background

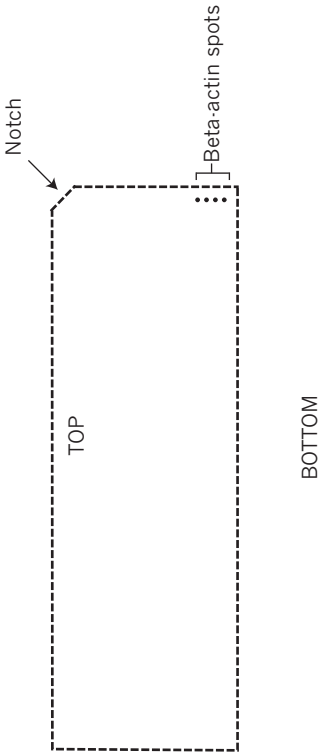
- ***Was the membrane completely immersed in solution during the incubations?***
  Never let the membrane dry out, and be sure membrane is completely immersed during all incubations. If desired, paper clips can be used to clip membranes to the bottom of the plastic containment during washing/blocking/detection steps. Use extreme caution when using this method.
- ***Was the membrane completely covered in the solutions during detection?***
  Be sure solutions completely cover the membrane during these steps.
- ***Were the hybridization solutions completely dissolved?***
  Check Hybridization Buffer for visible precipitated particles.
- ***Is the film overexposed?***
  Try several exposures for varying lengths of time. This is necessary for obtaining optimal results.
- ***How much antibody did you add to the Blocking Buffer (in Step E3)?***
  Make sure the antibody is diluted 1:5,000.

B. Problem: hybridization signals weak or absent

- ***Is the problem with your RNA quality?***
  Be sure to check the quality of your RNA (Section IV.A).
- ***Are the kit reagents working properly?***
  Strip the membrane and use the control K562 RNA (provided) to confirm that reagents and materials are working properly.
- ***Did you hybridize the membrane at the correct temperature?***
  Check to make sure the hybridization temperature is set at 52°C.
- ***How long was the hybridization time?***
  An overnight hybridization (~12 hours) is necessary for optimal binding of the probe to the array.
- ***How much CDP-Star substrate did you use?***
  Make sure that the substrate is evenly distributed over the membrane during detection (Step E5).
- ***How long was the exposure?***
  Try several exposures for varying lengths of time. This is necessary for obtaining optimal results.

VI. APPENDIX

Array Orientation



VI. APPENDIX *continued*

Solution/Dilution Recipes (enough for two array membranes)

250 ml of 2X SSC/0.5% SDS
To 212.5 ml of deionized H2O, add 25 ml of 20X SSC stock solution and 12.5 ml of 10% SDS stock solution. Shake well before use.

250 ml of 0.1X SSC/0.5% SDS
To 236.25 ml of deionized H2O, add 1.25 ml of 20X SSC stock solution and 12.5 ml of 10% SDS stock solution. Shake well before use.

75 ml of 1X Blocking Buffer
To 60 ml of deionized H2O, add 15 ml of 5X Blocking Buffer stock solution. Shake well before use.

200 ml of 1X Antibody Wash Buffer I
To 180 ml of deionized H2O, add 20 ml of 10X Antibody Wash Buffer I stock solution. Shake well before use.

200 ml of 1X Antibody Wash Buffer II
To 180 ml of deionized H2O, add 20 ml of 10X Antibody Wash Buffer II stock solution. Shake well before use.

100 ml of 1X Detection Buffer
To 90 ml of deionized H2O, add 10 ml of 10X Detection Buffer stock solution. Shake well before use.

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