

Oncogene Profiler Array Nonradioactive System

FOR RESEARCH USE ONLY

CATALOG #: 05-101

Description: The Oncogene Profiler™ Array Nonradioactive System employs digoxigenin (DIG)-11-dUTP-labeled cDNA and chemiluminescent detection. Each Oncogene Profiler Array contains 295 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 cancer and tumorigenesis. Enough reagents are provided for four hybridizations.

Package Contents

Profiler Array and Hybridization Kit:

2	Oncogene 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
15 µl	Random Primer Mix
15 µl	dNTP Mix
100 µl	dH2O

Profiler Array Chemiluminescence Detection Kit:

15 ml	CDP- <i>Star</i> 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

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.

(PAC0078)

Oncogene Profiler Gene List

CONTROLS	Coordinates	Genbank#
beta-actin	A1 & 19; P1, 10, 19 & 24 M24, N24, O24	X00351, J00074, M10278
Ubiquitin	B1 & 19; P2 & 11	M26880

The following gene list is ordered alphabetically for your convenience.

Gene	Coord.	Genbank #	Gene name
101F6	C01	AF040704	Homo sapiens putative tumor suppressor protein (101F6)
123F2	D01	AF040703	Homo sapiens putative tumor suppressor protein (123F2)
Abl-2	E01	NM_005158	Human v-abl Abelson murine leukemia viral oncogene homolog 2 (arg, Abelson-related gene) (ABL2) gene
AF-4	F01	X83606	H.sapiens AF-4 gene
Akt1	G01	NM_005163	Human v-akt murine thymoma viral oncogene homolog 1
Akt2	H01	NM_001626	Homo sapiens v-akt murine thymoma viral oncogene homolog 2
Akt3	I01	AF135794	Human AKT3 protein kinase
alpha-catenin	J01	S78736	CTNNA1=tumor suppressor gene alpha-catenin
aml-1	K01	L34598	Homo sapiens (clone PEBP2aB) aml1 (acute myeloid leukemia 1) oncogene (AML1)
APC	L01	M74088	Human APC gene
ARHA	M01	L09159	Homo sapiens RHOA proto-oncogene multi-drug-resistance protein mRNA, 3' end
AXL	N01	M76125, NM_061699	Human tyrosine kinase receptor (axl)
BAM22	O01	L13939	Homo sapiens beta adaptin (BAM22)
BARD1	A02	NM_000465	Homo sapiens BRCA1 associated RING domain 1 (BARD1)
BCD-ORF1	B02	U51869	Human proto-oncogene Bcd orf1 and orf2
BCD-ORF2	C02	U51869	Human proto-oncogene Bcd orf1 and orf2
Bcl2	D02	M14745	Human bcl-2
BCL3	E02	M31732	Human B-cell lymphoma 3-encoded protein (bcl-3)
Bcr	F02	NM_004327	Human breakpoint cluster region (BCR) gene
BECN1	G02	AF139131	Homo sapiens beclin 1 (BECN1) mRNA
BIN1	H02	AF068916	Homo sapiens bridging-integrator protein-1 isoform BIN1-13
BLYM	I02	K01884	Human Blym-1 transforming gene
Bmi1	J02	NM_005180	Homo sapiens murine leukemia viral (bmi-1) oncogene homolog (BMI1)
BRCA1	K02	NM_000058	Human breast cancer 1
BRCA2	L02	U43746	Human breast cancer 2
Brush-1	M02	S69790	Brush-1=tumor suppressor {3' region} [human, breast epithelium, mRNA Partial, 1485 nt]
C1ORF1	N02	NM_001213	Homo sapiens chromosome 1 open reading frame 1(C1ORF1)
c-abl	O02	NM_005157	Homo sapiens v-abl Abelson murine leukemia viral oncogene homolog 1 (Abl-1)
CAN	A03	X64228 S89710	H.sapiens can
CAV1	B03	NM_001753	Homo sapiens caveolin 1 (CAV)
CBL (c-cbl)	C03	X57110	Human c-cbl proto-oncogene
CBL-B	D03	U26710	Human cbl-b
CDC23	E03	AF053977	Homo sapiens cell division cycle protein 23 (CDC23)
CDH1	F03	Z13009	H.sapiens mRNA for E-cadherin
Cdk4	G03	NM_000075	Human cyclin-dependent kinase 4 (CDK4)
CHES1	H03	U68723	Human checkpoint suppressor 1
Ciao1	I03	U63810	Homo sapiens WD40 protein Ciao 1
c-mer	J03	NM_006343	Homo sapiens c-mer proto-oncogene tyrosine kinase (MERTK)
Cot	K03	D14497	Human mRNA for proto-oncogene protein
CREBL2	L03	NM_001310	Homo sapiens cAMP responsive element binding protein-like 2

Gene	Coord.	Genbank #	Gene name
CRK (c-crk)	M03	NM_005206	Human v-crk avian sarcoma virus CT10 oncogene homolog (CRK) gene
CRK-II	N03	D10656	Human mRNA for CRK-II
CRKL	O03	X59656	H.sapiens crk-like gene CRKL
Cyclin D1	P03	M64349	Human cyclin D1
Cyclin D2	A04	NM_001759	Human cyclin D2 (CCND2)
Cyclin D3	B04	M90814	Human D3-type cyclin (CCND3) gene
D10S170	C04	S72869	H4(D10S170)=putative cytoskeletal protein
DACH	D04	AF102546	Homo sapiens dachshund (DACH) mRNA
DAL1	E04	AF069072	Homo sapiens putative lung tumor suppressor (DAL1) mRNA
dbl	F04	X12556	Human dbl proto-oncogene
DCC	G04	X76132	Human DCC gene
DDX1	H04	NM_004939	Homo sapiens DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 1 (DDX1)
DDX6	I04	D17532	Human mRNA for RCK
dek	J04	X64229 S89712	H.sapiens dek mRNA
DLG2	K04	X82895	H.sapiens mRNA for DLG2
Doc-1	L04	AF006484	Homo sapiens putative oral tumor suppressor protein (doc-1)
Doc-1R	M04	NM_005851	Homo sapiens tumor suppressor deleted in oral cancer-related 1
DPC4 (SMAD4)	N04	U44378	Human homozygous deletion target in pancreatic carcinoma
DPH2L	O04	S81752	DPH2L=candidate tumor suppressor gene {ovarian cancer critical region of deletion}
E1Ac	P04	M18288	Adenovirus type 40 E1A and E1B genes
E1B19K	A05	M18288	Adenovirus type 40 E1A and E1B genes
E1B55K	B05	M18288	Adenovirus type 40 E1A and E1B genes
E2F-1	C05	M96577	Human (E2F-1) pRB-binding protein gene
ear3	D05	X12795	Human v-erbA related ear-3 gene
EGFR	E05	NM_005228	Human epidermal growth factor receptor
Egr 1	F05	NM_001964	Human early growth response 1 (EGR1) gene
EIF3S6	G05	U62962	Human Int-6 mRNA
EIF4B	H05	NM_001417	Human eukaryotic translation initiation factor 4B (EIF4B)
ELE1	I05	X71413	H.sapiens ELE1 mRNA
ELK1	J05	M25269	Homo sapiens tyrosine kinase (ELK1) oncogene mRNA
ELK3	K05	Z36715	H.sapiens mRNA for Net transcription factor
EMP1	L05	U77085	Human epithelial membrane protein (CL-20) mRNA
EMS1	M05	M98343	Homo sapiens amplaxin (EMS1) mRNA
EPHA1	N05	M18391	Human tyrosine kinase receptor (eph) mRNA
EPHA3	O05	M83941	Human receptor tyrosine kinase (HEK) mRNA
ERBAL2	P05	X12794	Human v-erbA related ear-2 gene
ERbB2 (c-erbB2)	A06	X03363	Human c-erb-B-2 mRNA.
ERBB3	B06	M34309	Human epidermal growth factor receptor (HER3) mRNA
ERBB4	C06	L07868	Homo sapiens receptor tyrosine kinase (ERBB4) gene
Ets1	D06	J04101	Human erythroblastosis virus oncogene homolog 1 (ets-1)
Ets2	E06	NM_005239	Homo sapiens v-ets avian erythroblastosis virus E26 oncogene homolog 2 (ETS2) mRNA.
ETV3	F06	L16464	Human ETS oncogene (PEP1) mRNA
ETV6	G06	U11732	Human ets-like gene (tel) mRNA
EV1	H06	S82592	Evi-1=Evi-1 protein {3' region, deletion region}
EWSR1	I06	X66899	H.sapiens EWS mRNA
EXT2	J06	NM_000401	Homo sapiens exostosins (multiple) 2 (EXT2) mRNA
EXTL2	K06	AF000416	Homo sapiens EXT-like protein 2 (EXTL2) mRNA
EXTL3	L06	AF083551	Homo sapiens exostosin (EXTL3) mRNA
FAT	M06	X87241	H.sapiens mRNA for hFat protein
FER	N06	J03358	Human tyrosine kinase (FER) mRNA
FES (c-fes)	O06	X52192	Human c-fes/fps proto-oncogene
FGF-3	P06	NM_005247	Human fibroblast growth factor 3 (murine mammary tumor virus integration site (v-int-2) oncogene homolog) (FGF3)

Gene	Coord.	Genbank #	Gene name
FGF-4	A07	NM_002008	Human fibroblast growth factor 4 (heparin secretory transforming protein 1, Kaposi sarcoma oncogene) (FGF4)
FGF-6	B07	X63454	H.sapiens hst-2 (FGF-6) mRNA
FGFR	C07	M34185	Human fibroblast growth factor receptor (FGFR) transmembrane form mRNA, complete cds
FGR	D07	M19722 J03429	Human Proto-oncogene
FHIT	E07	NM_002012	Human fragile histidine triad gene (FHIT)
FKHL1	F07	X74142	H.sapiens HBF-1 mRNA for transcription factor
FLI-1	G07	X67001, S44250	H.sapiens HUMFLI-1 mRNA
FLT-1	H07	AF063657	Homo sapiens vascular endothelial growth factor receptor
Flt-1	I07	AF063657	Homo sapiens vascular endothelial growth factor receptor (FLT1) mRNA
Flt3	J07	Z26652, S64785	H.sapiens FLT3 mRNA for FLT3 receptor tyrosine kinase
Flt4	K07	X68203	H.sapiens mRNA for FLT4, class III receptor tyrosine kinase
FMS (c-fms)	L07	X03663	Human c-fms proto-oncogene
fos (c-fos)	M07	K00650 M16287	Human Proto-oncogene
FOSL1	N07	X16707	Human fra-1 mRNA
FOSL2	O07	X16706	Human fra-2 mRNA
FRAT1	P07	U58975	Homo sapiens proto-oncogene (FRAT1) mRNA
FRAT2	A08	AF062739	Homo sapiens GSK-3 binding protein FRAT2 (FRAT2)
Fus-2	B08	AF040706	Homo sapiens putative tumor suppressor protein (Fus-2)
Gene 21	C08	AF040708	Homo sapiens candidate tumor suppressor gene 21 protein
Gene 26	D08	AF040709	Homo sapiens putative tumor suppressor gene 26 protein alpha 2 delta calcium channel subunit Mrna
gli	E08	NM_005269	Homo sapiens glioma-associated oncogene homolog (zinc finger protein) (GLI) mRNA.
gli2	F08	NM_005270	Homo sapiens GLI-Kruppel family member GLI2 (GLI2)
GLI3	G08	M57609, M34366	Human DNA-binding protein (GLI3) mRNA
GRO1	H08	X12510	Human mRNA for melanoma growth stimulatory activity (MGSA).
HCK	I08	M16591	Human hemopoietic cell protein-tyrosine kinase (HCK) gene
hdlg-1	J08	U13897	Human homolog of Drosophila discs large protein, isoform 1
HEMK	K08	AF131220	Homo sapiens HEMK homolog (HEMK) mRNA
HLF	L08	M95585	Human hepatic leukemia factor (HLF) mRNA
Hox11	M08	NM_005521	Homo sapiens homeo box 11 (T-cell lymphoma 3-associated breakpoint) (HOX11) mRNA
HPV E6	N08	K02718	Human papillomavirus type 16 (HPV16), complete genome
HPV E7	O08	K02718	Human papillomavirus type 16 (HPV16), complete genome
hsReg	P08	U94585	Homo sapiens requiem homolog (hsReq) mRNA
ING1	A09	AF110645	Human candidate tumor suppressor p33 ING1 homolog gene
ING1L	B09	NM_001564	Homo sapiens inhibitor of growth 1-like (ING1L) mRNA
IRF-4	C09	U52682	Human lymphocyte specific interferon regulatory factor/interferon regulatory factor 4 (LSIRF/IRF4) mRNA
JAW-1	D09	U10485	Human lymphoid-restricted membrane protein (Jaw1) mRNA
Jun (c-jun)	E09	J04111	Human c-jun proto oncogene
JunB	F09	X51345	Human jun-B mRNA for JUN-B protein.
JunD	G09	X51346	Human jun-D mRNA for JUN-D protein
KAI1	H09	U20770	Human metastasis suppressor (KAI1) mRNA
Kit (c-kit)	I09	X06182	Human Proto-oncogene
LAF4	J09	NM_002285	Homo sapiens lymphoid nuclear protein related to AF4 (LAF4)
Large T Ag	K09	J02400	Simian virus 40 Large T Antigen
LATS1	L09	NM_004690	Homo sapiens LATS (large tumor suppressor, Drosophila) homolog 1 (LATS1) mRNA
LBC	M09	NM_006738	Homo sapiens lymphoid blast crisis oncogene (LBC), mRNA
LCK (c-Lck)	N09	NM_005356	Homo sapiens lymphocyte-specific protein tyrosine kinase
LEU1	O09	Y15227	Homo sapiens mRNA for leukemia associated gene 1
Leu2	P09	Y15228	Homo sapiens mRNA for leukemia associated gene 2
Leu5	A10	NM_005798	Homo sapiens candidate tumor suppressor involved in B-CLL

Gene	Coord.	Genbank #	Gene name
LIBC	B10	AF143679	Homo sapiens lost in inflammatory breast cancer tumor suppressor protein (LIBC) mRNA
LUCA-1	C10	U03056	Homo sapiens putative tumor suppressor (LUCA1/HYAL1)
LUCA15	D10	U23946	Human putative tumor suppressor (LUCA15) mRNA
LUCA-2	E10	NM_003773	Homo sapiens hyaluronoglucosaminidase 2 (HYAL2) mRNA.
LUCA-3	F10	AF040710	Homo sapiens hyaluronidase (LUCA-3) mRNA
M1S1	G10	X77753	H.sapiens TROP-2 gene
M4S1	H10	M32325	Human adenocarcinoma-associated antigen (KSA) mRNA
mad	I10	L06895	Human antagonist of myc transcriptional activity
Maf (c-maf)	J10	NM_005360	Human v-maf musculoaponeurotic fibrosarcoma (avian) oncogene
MAFB	K10	AF134157	Homo sapiens MAFB/Kreisler basic region/leucine zipper transcription factor (MAFB) mRNA
MAFG	L10	U84249	Homo sapiens basic-leucine zipper transcription factor MafG
MAPKAPK3	M10	NM_004635	Homo sapiens mitogen-activated protein kinase-activated protein kinase 3 (MAPKAPK3) Mrna
Mas (c-mas)	N10	M13150	Human mas proto-oncogene gene
MASL1	O10	NM_004225	Homo sapiens MFH-amplified sequences with leucine-rich tandem repeats 1 (MASL1), mRNA
max	A11	X60287, S95058	Human max gene
MCC	B11	M62397	Human colorectal mutant cancer protein mRNA
MDA-7	C11	NM_006850	Homo sapiens suppression of tumorigenicity 16 (melanoma differentiation) (MDA-7), mRNA
MDM2	D11	Z12020	Human p53-associated gene
MEL	E11	S53268	MEL=RAS-related [human, Genomic/mRNA, 1980 nt].
MEN1	F11	U93236	Human menin (MEN1) gene
Met (c-met)	G11	M60718	Human hepatocyte growth factor gene
MKK4	H11	U17743	Human JNK activating kinase (JNKK1) mRNA
MLH1	I11	U07343	Human DNA mismatch repair protein homolog (hMLH1)
MN1	J11	NM_002430	Homo sapiens meningioma (disrupted in balanced translocation) 1 (MN1) mRNA
Mos (c-mos)	K11	J00119	Human humos gene homologous to transforming gene of mmsv
Mpl (c-mpl)	L11	M90102	Human (clones 15, 39, 41) c-myceloproliferative leukemia virus type P (c-mpl-P) mRNA
MRV11	M11	NM_006069	Homo sapiens murine retrovirus integration site 1 homolog
MSH2	N11	U04045	Human (hMSH2) mRNA
MTCP-1	O11	Z24459, Z24460	H.sapiens MTCP1 gene, exons 2A to 7 (and joined mRNA).
MX11	A12	NM_005962	Homo sapiens MAX-interacting protein 1 (MX11) mRNA
Myb (b-myb)	B12	X13293	Human B myb oncogene
Myb (c-myb)	C12	M15024	Human Proto-oncogene
MYBL1	D12	X66087	H.sapiens a-myb mRNA
Myc (c-myc)	E12	E01841, J00120	Human proto-oncogene
Myc (L-myc)	F12	M19720	Human L-myc protein gene
Myc (n-myc)	G12	Y00664	Human germ line n-myc gene
NBL1	H12	D28124	Human mRNA for unknown product
NE-dlg	I12	U49089	Human neuroendocrine-dlg (NE-dlg) mRNA
NEO1	J12	U61262	Human neogenin mRNA
NF1	K12	M89914	Human neurofibromin (NF1) gene
NF2	L12	NM_000268	Homo sapiens neurofibromatosis type 2 (NF2) mRNA
NFkB2	M12	X61498	H.sapiens mRNA for NF-kB subunit
NKTR	N12	L04288	Homo sapiens cyclophilin-related protein mRNA
NOC2	O12	AF129812	Homo sapiens candidate tumor suppressor protein NOC2
NOEY2	P12	U96750	Homo sapiens putative tumor suppressor NOEY2 mRNA
NOV	A13	X96584	H.sapiens mRNA for NOV protein
novH	B13	X78351	H.sapiens novH gene exons 1 and 2
TPR	C13	X66397	H.sapiens tpr mRNA

Gene	Coord.	Genbank #	Gene name
ODC	D13	M34158, J05271	Human ornithine decarboxylase (ODC) gene
p107	E13	L14812	Human retinoblastoma related protein (p107) gene
p15	F13	L36844	Human (clone p15INK4B/HA5) CDK inhibitory protein gene
p16	G13	L27211	Human CDK4-inhibitor (p16-INK4) gene
p21	H13	L25610	Human cyclin-dependent kinase inhibitor mRNA
p53	I13	K03199	Human p53 Tumor Antigen
p57	J13	U48869	Human cdk-inhibitor p57/KIP2 (CDKN1C) gene
p73	K13	Y11416	H.sapiens mRNA for P73
PAR-4	L13	NM_002583	Homo sapiens apoptosis response protein (PAWR) mRNA
PAX2	M13	M89470	Human paired-box protein (PAX2) mRNA
PBX1	N13	NM_002585	Homo sapiens pre-B-cell leukemia transcription factor 1
PBX2	O13	X59842	Human PBX2 mRNA
PBX3	P13	X59841	Human PBX3 mRNA
PDGF-b (c-sis)	A14	X02811	Human platelet-derived growth factor B chain (PDGF-B) gene
PDGFRL	B14	NM_006207	Homo sapiens platelet-derived growth factor receptor-like
PEP1	C14	L16464	Human ETS oncogene (PEP1) mRNA
PFM1	D14	AF144757	Homo sapiens PR-domain zinc-finger protein PFM1 mRNA
Pim (c-Pim2)	E14	NM_006875	Homo sapiens proto-oncogene Pim-2 (serine threonine kinase)
Pim-1	F14	NM_002648	Human pim-1 oncogene (PIM1) gene
PLA2P	G14	AF145020	Homo sapiens phospholipase A2 activating protein (PLA2P)
PP32R1	H14	AF008216	Homo sapiens candidate tumor suppressor pp32r1 (PP32R1)
PRG1	I14	X96438	H.sapiens PRG1 gene
PRLTS	J14	D37965	Human mRNA for PDGF receptor beta-like tumor suppressor
PTC	K14	U43148	Human patched homolog (PTC) mRNA
PTC-CP	L14	M31213	Human papillary thyroid carcinoma-encoded protein mRNA
PTCH	M14	U43148	Human patched homolog (PTC) mRNA
PTEN	N14	U93051	Human putative protein tyrosine phosphatase (PTEN) gene
PTI-1	O14	L41498	Homo sapiens longation factor 1-alpha 1 (PTI-1) mRNA
PTPG1	P14	S69184	PTPG1=tumor suppressor {3' region, clone DC1} [human, colo-rectal carcinoma cell line DLD-1, mRNA Partial Mutant, 236 nt].
PVT1	A15	X04620	Human lymphoma pvt-1 locus DNA near t(2;8) translocation breakpoint
A-Raf-1	B15	X04790	Human mRNA for A-raf-1 oncogene
Raf (c-raf1)	C15	X03484	Human mRNA for raf oncogene
Raf-b	D15	NM_004333	Homo sapiens v-raf murine sarcoma viral oncogene homolog B1 (BRAF)
Ral (c-ral A)	E15	NM_005402	Homo sapiens v-ral simian leukemia viral oncogene homolog A (ras related) (RALA), mRNA.
RalB	F15	NM_002881	Homo sapiens v-ral simian leukemia viral oncogene homolog B (ras related; GTP binding protein) (RALB), mRNA
RAN	G15	NM_006325	RAN, member RAS oncogene familyHomo sapiens RAN, member RAS oncogene family (RAN), mRNA
RARRES3	H15	AF060228	Homo sapiens retinoic acid receptor responder 3 (RARRES3)
Ras (Ha-ras)	I15	M30708	Synthetic human c-Ha-ras gene encoding p21 protein
Ras (Ki-ras)	J15	L00044, K00653	Human cellular c-Ki-ras2 proto-oncogene
Ras (N-ras)	K15	M14307	Synthetic N-ras gene
Rb (p110)	L15	M28419	Human retinoblastoma susceptibility protein mRNA
Rb2 (p130)	M15	X74594	Human Rb2/p130 protein
RBX1	N15	AF140598	Homo sapiens ring-box protein 1 (RBX1) mRNA
RDA32	O15	AF061836	Homo sapiens putative tumor suppressor protein (RDA32)
RECK	P15	E13833	Human tumor suppressor gene,RECK gene
Rel (c-rel)	A16	NM_002908	Homo sapiens v-rel avian reticuloendotheliosis viral oncogene homolog (REL) mRNA.
Ret	B16	X12949, M57464	Human ret proto-oncogene for tyrosine kinase
RhoA	C16	L25080	Human GTP-binding protein (rhoA)
RON	D16	X70040	H.sapiens RON mRNA for tyrosine kinase

Gene	Coord.	Genbank #	Gene name
Ros (c-ros1)	E16	NM_002944	Homo sapiens v-ros avian UR2 sarcoma virus oncogene homolog 1 (ROS1) mRNA.
shb	F16	X75342	H.sapiens SHB mRNA
shc	G16	U73377	Human p66shc (SHC) mRNA
Ski (c-ski)	H16	X15218	Human ski oncogene gene
Smad4	I16	U44378	Human homozygous deletion target in pancreatic carcinoma (DPC4) gene
SMAD5	J16	U73825	Homo sapiens Smad5 mRNA
SMARCB1	K16	Y17118	Homo sapiens SNF5/IN11 gene, exon 1 (and joined CDS).
SnoN	L16	X15219	Human sno oncogene mRNA for snoN protein, ski-related
SP1	M16	X52056	Human mRNA for spi-1 proto-oncogene
SPINK1	N16	Y00705	Homo sapiens pstI mRNA for pancreatic secretory inhibitor (expressed in neoplastic tissue)
src (c-fyn)	O16	NM_002037	Human FYN oncogene related to SRC, FGR, YES (FYN)
src (c-Lyn)	P16	M16038	Human lyn mRNA encoding a tyrosine kinase
Src (c-src)	A17	X59932, X71157	Human C-SRC-kinase
SRP-1	B17	U20620	Homo sapiens SRP1 mRNA, partial sequence
ST13	C17	U17714	Homo sapiens putative tumor suppressor ST13 (ST13) mRNA
ST5	D17	S45936	HTS1=HeLa tumor suppressor gene [human, revertant clone F2, mRNA Partial, 2687 nt]
SUPT3H	E17	AF064804	Homo sapiens transcription factor SUPT3H (SUPT3H)
SUPT5H	F17	U56402	Human chromatin structural protein homolog (SUPT5H)
SUPT6H	G17	U46691	Human putative chromatin structure regulator (SUPT6H)
TAL1	H17	M29038	Human stem cell protein (SCL) mRNA
TCL-1	I17	X82240	H.sapiens mRNA for Tcell leukemia/lymphoma 1
TCL-3	J17	M62626	Human T lymphocyte homeobox protein (partial cds)
TEF	K17	U06935	Human thyrotroph embryonic factor (TEF) mRNA
TEL	L17	U11732	Human ets-like gene (tel) mRNA
TGFb-RII	M17	NM_003242	Homo sapiens transforming growth factor, beta receptor II (70-80kD) (TGFB2) mRNA
THRA	N17	M24748	Human thyroid hormone receptor alpha 1 (TR-alpha-1) gene
THRB	O17	M26747	Human c-erbA mRNA
TIAM1	P17	U16296	Human T-lymphoma invasion and metastasis inducing TIAM1 protein (TIAM1) mRNA
TID1	A18	NM_005147	Homo sapiens tumorous imaginal discs (Drosophila) homolog (TID1) mRNA
Tim	B18	NM_005435	Human Oncogene TIM (TIM) gene
TIMP-3 (HUMAN)	C18	U14394	Human tissue inhibitor of metalloproteinases-3 mRNA
TLN	D18	NM_006289	Homo sapiens talin (TLN) mRNA
TM4SF1	E18	M90657	Human tumor antigen (L6) mRNA
TNF	F18	X01394	Human tumor necrosis factor
TNF-b	G18	D12614	Human mRNA for lymphotoxin (TNF-beta),
TNF-c	H18	L11015	Homo sapiens lymphotoxin-beta mRNA
TP53BP2	I18	U58334	Human Bcl2, p53 binding protein Bbp/53BP2 (BBP/53BP2)
TPO	J18	NM_000460	Human thrombopoietin (myeloproliferative leukemia virus oncogene ligand, megakaryocyte growth and development factor) (THPO) gene
TreORF1 (210)	K18	X63546	H.sapiens mRNA for tre oncogene (clone 210).
TreORF2 (213)	L18	X63547	H.sapiens mRNA for tre oncogene (clone 213).
TSC1	M18	AF013168	Homo sapiens hamartin (TSC1) mRNA
UNPH	N18	U20657	Human ubiquitin protease (Unph) proto-oncogene mRNA, complete cds
USP4	O18	NM_003363	Homo sapiens ubiquitin specific protease 4 (proto-oncogene) (USP4) mRNA
vav	P18	X16316	Human vav oncogene
VAV2	C19	NM_003371	Homo sapiens vav 2 oncogene (VAV2) mRNA

Gene	Coord.	Genbank#	Gene name
VAV3	D19	NM_006113	Homo sapiens vav 3 oncogene (VAV3) mRNA
VHL	E19	NM_000551	Homo sapiens von Hippel-Lindau syndrome (VHL) mRNA
v-src	F19	D10652	Rous sarcoma virus genome
wnt-1	G19	NM_005430	Human wingless-type MMTV integration site family, member 1 (WNT1) gene
WNT2	H19	X07876, Y00838	Human mRNA for irp protein (int-1 related protein).
WNT5	I19	L20861	Homo sapiens proto-oncogene (Wnt-5a) mRNA
Wnt7a	J19	D83175	Homo sapiens WNT7a mRNA
WT	K19	X51630	Human Wilms tumor WT1 mRNA for zinc finger protein, Krueppel-like
Yes (c-yes)	L19	M15990	Human c-yes-1

NOTES

NOTES

Trademark

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**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.

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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*

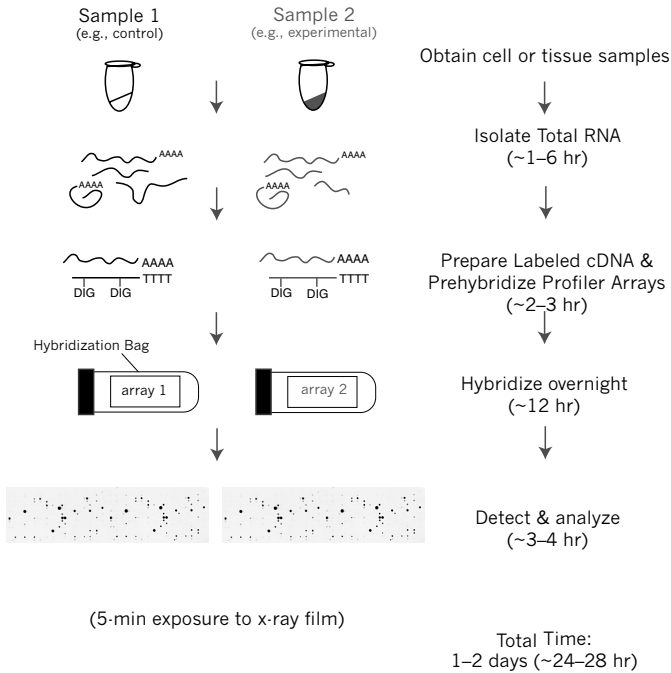


Figure 1 provides a simplified overview of the Profiler procedure. After cDNA labeling, Profiler Arrays are hybridized overnight with DIG-11-dUTP-labeled cDNA. The next day, the array membranes are washed and subsequently incubated with Anti-DIG-Alkaline Phosphatase (DIG-AP) Antibody. Upon addition of 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 dH₂O (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*

- 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.

- 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.

- 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

- Mix well by pipetting. Incubate the cDNA synthesis mixture at 42°C for 2 min.
- 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.
- 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:

- 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.
- 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.
- 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 μ l pipet tip container. Quickly 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 μ l 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

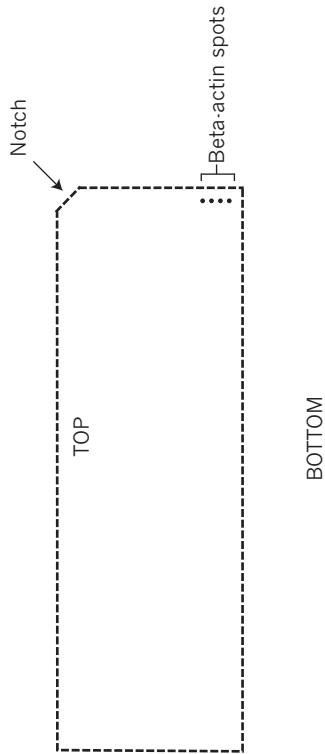
- ***Was the membrane completely immersed in solution during the incubations?***
Never let the membrane dry out, and be sure membrane is completely immersed during all incubations. If desired, paper clips can be used to clip membranes to the bottom of the plastic containment during washing/blocking/detection 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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