Human Cancer Drug Target qPCR Array

Catalogue # GA-C118A, GA-C118B

Description

Our PCR Array plates are pre-coated with EvaGreen-optimized primer assays for a thoroughly researched panel of relevant, pathway- or disease-focused genes. Our **unique high-quality primer design and master mix formulation** enable the PCR Array to amplify 96 different gene-specific products simultaneously under uniform cycling conditions. All primer sets designed by our expertise scientists are able to amplify the **alternative splice variants** of corresponding target genes.

This Human Cancer Drug Target Array is designed to profile the expression of **88 key genes or targets for anticancer therapeutics and drug development**.

Features

- **High Sensitivity**: cDNA made from as little as 1 ng (or as much as 5 µg) of total RNA per array plate provides greater than 85 percent present call rates.
- **High Reproducibility**: the system has replicate correlation coefficients > 0.99, which means that experimental samples can be reliably compared across plates and runs.
- **High Specificity**: the combination of EvaGreen primers and 2x Elite™ qPCR MasterMix guarantees a single product of the predicted size from every reaction without secondary products such as primer dimers. Controls are also included for monitoring genomic DNA contamination, RNA quality, and general PCR performance.
- **Easy to Use**: simple experiment workflow and easy-to-use Excel-based template for data analysis. The analysis is based on the $\Delta\Delta C_t$ method with normalization of the raw data to either housekeeping genes or an external RNA control. This PCR Array is compatible with, but not limited to, all ABI, Bio-Rad, Eppendorf, QIAGEN, Roche, and Stratagene instruments.

Kit Components

- 2x Elite™ HotStart qPCR MasterMix (HotStart Taq, dNTP, EvaGreen Dye; ROX Passive Reference Dye included for format B)
- Adhesive films (1 piece each plate)
- Manual and PCR Data Analysis Tool (one CD included)
- 96-well plate array (see the table below for the genes included)

A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	A12
ABCC1	*AKT1*	*AKT2*	*ATF2*	*AURKA*	*AURKB*	*AURKC*	*BCL2*	*BIRC5*	*CDK1*	*CDC25A*	*CDK2*
B01	B02	B03	B04	B05	B06	B07	B08	B09	B10	B11	B12
CDK4	*CDK5*	*CDK7*	*CDK8*	*CDK9*	*CTSB*	*CTSD*	*CTSL1*	*CTSS*	*EGFR*	*ERBB2*	*ERBB3*
C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12
ERBB4	*ESR1*	*ESR2*	*FIGF*	*FLT1*	*FLT4*	*GRB2*	*GSTP1*	*HDAC1*	*HDAC11*	*HDAC2*	*HDAC3*
D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12
HDAC4	*HDAC6*	*HDAC7*	*HDAC8*	*HIF1A*	*HRAS*	*HSP90AA1*	*HSP90B1*	*IGF1*	*IGF1R*	*IGF2*	*IRF5*
E01	E02	E03	E04	E05	E06	E07	E08	E09	E10	E11	E12
KDR	*KIT*	*KRAS*	*MDM2*	*MDM4*	*MTOR*	*NFKB1*	*NRAS*	*NTN3*	*PARP1*	*PARP2*	*PARP4*
F01	F02	F03	F04	F05	F06	F07	F08	F09	F10	F11	F12
PDGFRA	*PDGFRB*	*PGR*	*PIK3C2A*	*PIK3C3*	*PIK3CA*	*PLK1*	*PLK2*	*PLK3*	*PLK4*	*PRKCA*	*PRKCB*
G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12
PRKCD	*PRKCE*	*PTGS2*	*RHOA*	*RHOB*	*TERT*	*TNKS*	*TOP2A*	*TOP2B*	*TP53*	*TXN*	*TXNRD1*
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
TP73	*YY1*	*RPL13A*	*B2M*	*HGD1*	*HGD*	*GAPDH*	*GAPDH*	*ACTB*	*ACTB*	*tuba1b*	*HPRT1*

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Order Information

We have two formats of 2x Elite™ qPCR MasterMix for different type of the realtime thermal cyclers.

- **Format A** is suitable for use with the real-time thermal cyclers that do not require a reference dye:

Bio-Rad models CFX96, CFX384;
Bio-Rad/MJ Research models Chromo4, DNA Engine Bio-Rad models iCycler, iQ5, MyiQ, MyiQ2, Opticon 2;
Roche LightCycler 480 (96-well).

qPCR Array Format A	Human Cancer Drug Target qPCR Array trial size (***Cat# GA-C118A1***)	Human Cancer Drug Target qPCR Array (***Cat# GA-C118A***)
96-Well Plate Containing Dried Assays ***(Part# C118-120)***	2 plates	12 plates
Adhesive Film ***(Part# GA-005)***	2 pieces	12 pieces
2x Elite™ qPCR MasterMix (HotStart Taq, dNTP, EvaGreen Dye) ***(Part# GA-135)***	2 x 1.25 ml	12x 1.25 ml

- **Format B** is suitable for use with the following real-time thermal cyclers:

Applied Biosystems models 5700, 7300, 7500 (Standard and Fast), 7700, 7900HT (Standard and Fast), StepOnePlus, ViiA7 (Standard and Fast);
Eppendorf Mastercycler ep realplex models 2, 2S, 4, 4S;
Stratagene models Mx3000P, Mx3005P, Mx4000;
Takara TP-800.

qPCR Array Format B	Human Cancer Drug Target qPCR Array trial size (***Cat# GA-C118B1***)	Human Cancer Drug Target qPCR Array (***Cat# GA-C118B***)
96-Well Plate Containing Dried Assays ***(Part# C118-120)***	2 plates	12 plates
Adhesive Film ***(Part# GA-005)***	2 pieces	12 pieces
2x Elite™ qPCR MasterMix (HotStart Taq, dNTP, EvaGreen Dye, ROX Passive Reference Dye) ***(Part# GA-245)***	2 x 1.25 ml	12x 1.25 ml

Storage

Keep in freezer (-20 °C) and avoid exposure to light.

Materials Required But Not Included

- The Reverse transcription reagents for making the cDNA from your prepared total RNA are not included in the array kit (Protocol and reagents from Invitrogen and Qiagen for reverse transcription have been tested and worked well along with this kit).
- High-quality, nuclease-free water. Do not use DEPC-treated water
- Low EDTA–TE buffer (0.1 mM EDTA)

Important Notes before Use

1. Please read through this entire protocol before beginning your experiment.

2. The use of eEnzyme 2x Elite™ qPCR MastMix (included) is critical for obtaining the most accurate results from the PCR Array.
3. Make sure you have the correct PCR array plate format for your realtime PCR instrument to avoid damage.
4. The accuracy and precision of pipetting determines the consistency of the results. Make sure that all the micro-pipettors used are calibrated and not to introduce any bubbles into the wells of the PCR Array.
5. DEPC treated H_2O should **NOT** be used. Use high-quality, nuclease-free H_2O. Check with the supplier if not sure whether your RNase, DNase-free water has been treated with DEPC.
6. Exam the quality of your sample RNA before starting the experiment.
7. If precipitates are present in eEnzyme 2x Elite™ qPCR MastMix tubes, please contact a technical application scientist at 1-800-919-0755 or info@eenzym.com.
8. Regarding the concern of genomic DNA contamination: our arrays are designed to skip at least one intron so that traces of contaminated genomic DNA in the sample, if there is any, will not be amplified. In addition, each pair of primers are designed to have 60 ^{o}C±1 annealing temperature, which guarantees that large-sized genomic DNA, if any, cannot be amplified.

Workflow and Protocols

1. Make cDNA from your sample RNA.
   (refer to your reverse transcription kit manual, not included in the array kit.)
2. Thaw 2x Elite™ qPCR MasterMix on ice, vortex and briefly spin down.
3. Mix all following components in a tray for multi-channel pipetting. Carefully pipette precise 25 µl reaction mix to each of the 96 wells. Change pipet tips following each addition to avoid any cross-contamination.

2x Elite™ qPCR MasterMix	1250 µl
Diluted cDNA	100 µl
nuclease-free H_2O	1150 µl
Total Volume	2500 µl

   Note: save the remainder of the cDNA synthesis reaction and store at -20 ^{o}C for possible RNA quality analysis in later troubleshooting step.
4. Loading the PCR arrays:
   Please select your PCR Array Format for loading instruction.
   4.1 Carefully remove the PCR Array from its sealed bag.
   4.2 Dispense Experimental Cocktail to PCR Array Loading Reservoir to assist in loading (optional).
   4.3 Add 25 µl of the Experimental cocktail to each well of the PCR Array, preferably from a reservoir with an eight- or twelve-channel pipettor.
5. Performing realtime PCR detection:
   Attention: Users of Bio-Rad and Eppendorf Realtime instruments - prior to initiating the run, make sure your instrument has been calibrated for using clear sticky film.
   Note: follow the manufacturer's instruction for the proper operation and maintenance of your realtime instrument.
   5.1. Carefully and tightly seal the PCR Array with the optical thin adhesive film.
   5.2. Centrifuge the plate for 1 full minute at 4 ^{o}C at 1000g to remove bubbles. Visually inspect the plate from underneath of the plate to ensure no bubbles are present in each well.
   5.3. Place the plate on ice while setting up the PCR cycler program below.
   5.4. Place the plate in your realtime thermal cycler if recommended by your instrument's user manual, use a compression pad with the optical film-sealed plate formats.
      Note: PCR Arrays containing experimental cocktail may be store at -20 ^{o}C wrapped in aluminum foil for up to one week until ready to run.
   5.5. Enter and run the appropriate program for your realtime instrument. We provide a file to help customs easy to load software for both ABI and Bio-Rad realtime PCR instruments.

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Use a Two-step cycling program for the following instrumentation:

Real Time PCR Instruments	***Cycles***	***Duration***	***Temperature***
ABI:5700, 7000, 7300, 7500, 7700,7900HT	*1*	*5 min*	*95 °C*
StepOnePlus Bio-Rad: icycler, IQ5, MyiQ, MyiQ2, CFX96, CF384.	*40*	*15 seconds*	*95 °C*
Eplpendorf: Mastercycler ep realplex 2, 2s, 4, 4S			
Stratagene: Mx3000p, Mx3005p, Mx4000p		*1 min*	*58 °C*

Attention: *Bio-Rad CFx96 &CF384 users- adjust the ramp rate to 1 °C/sec.*

5.6. Calculate the threshold cycle (Ct) for each well using the instrument's software.

Note: for Roche Light Cycler 480 Users, there are two options available to analyze your data. Use the second derivate max setting and there is no need to set a threshold.

i. To define the Baseline. Choose the Automated Baseline option if your instrument has the Adaptive Baseline Function (check with instrument manual or manufacturer if unsure). If it does not have the adaptive baseline function, you will need to set the baseline manually. Use the Linear View of the amplification plots to determine the earliest visible amplification. Set the instrument to use the readings from cycle number two (2) through two (2) cycles before the earliest visible amplification, but no more than cycle 15. The earliest amplification usually will be visible between cycles 14 and 18.

ii. Manually define the threshold value by using the log view of the amplification plots and place it above the background signal but within the lower one-third to lower one half of the linear phase of the amplification plot.

Important: ensure that the thresholds are the same across all PCR Array runs in the same analysis. The absolute position of the threshold is less critical than its consistent position across arrays. When the quality of the RNA sample adequately controlled, the cycling program executed properly, and the thresholds defined correctly, the value of Ct^{ppc} should be 20±2 cross all of your arrays or samples.

iii. Export the resulting threshold cycle values for all wells to a blank Excel spreadsheet for use with the PCR Array Data Analysis Template Excel.

6. Recommended Quality Control: Dissociation (Melting) Curve
For instrument specific melt curve analysis settings, please refer to the corresponding instrument Setup Guide.

Note: If you decide not to obtain the dissociation curve immediately, save the plates in aluminum foil at -20 °C as is, in case you need to do this operation at a later time for troubleshooting. When ready, simply warm the plate to room temperature, place it into your realtime instrument, and run the melting program described above.

i. Be sure to visually inspect the plate after the run for any sign of evaporation from any of the wells. If evaporation is observed, make a note of which wells so that you may qualify your data analysis appropriately.

ii. Do not open any previously run and stored PCR Array plate. Removing the adhesive film to see if PCR product is evaporated during PCR process.

iii. Run a melting curve program immediately after the above cycling program, and generate a first derivative dissociation curve for each well in the entire plate using your instrument's software. No more than one peak should appear in each reaction at temperatures greater than 80 °C. If your instrument does not have a default melting curve program, run the following program instead: 95 °C 1min. 65 °C 2min (Optics off); 65 °C to 95 °C at 2 °C/min (Optics ON).

Gene Information

Position	GeneBank	Symbol	Name
A01	NM_004996.3	ABCC1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1
A02	NM_005163.2 NM_001014432.1 NM_001014431.1	AKT1	V-akt murine thymoma viral oncogene homolog 1
A03	NM_001626.4 NM_001243028.1 NM_001243027.1	AKT2	V-akt murine thymoma viral oncogene homolog 2
A04	NM_001880.3 NM_001256093.1 NM_001256091.1 NM_001256094.1 NM_001256092.1 NM_001256090.1	ATF2	Activating transcription factor 2
A05	NM_003600.2 NM_198437.1 NM_198436.1 NM_198435.1 NM_198434.1 NM_198433.1	AURKA	Aurora kinase A
A06	NM_004217.3 NM_001256834.1	AURKB	Aurora kinase B
A07	NM_003160.2 NM_001015879.1 NM_001015878.1	AURKC	Aurora kinase C
A08	NM_000633.2 NM_000657.2	BCL2	B-cell CLL/lymphoma 2
A09	NM_001168.2 NM_001012271.1 NM_001012270.1	BIRC5	Baculoviral IAP repeat containing 5
A10	NM_001786.4 NM_033379.4 NM_001170407.1 NM_001170406.1	CDK1	Cyclin-dependent kinase 1
A11	NM_001789.2 NM_201567.1	CDC25A	Cell division cycle 25 homolog A (S. pombe)
A12	NM_001798.3 NM_052827.2	CDK2	Cyclin-dependent kinase 2
B01	NM_000075.3	CDK4	Cyclin-dependent kinase 4
B02	NM_004935.3 NM_001164410.1	CDK5	Cyclin-dependent kinase 5
B03	NM_001799.3	CDK7	Cyclin-dependent kinase 7
B04	NM_001260.1	CDK8	Cyclin-dependent kinase 8
B05	NM_001261.3	CDK9	Cyclin-dependent kinase 9
B06	NM_001908.3 NM_147783.2 NM_147782.2 NM_147781.2 NM_147780.2	CTSB	Cathepsin B
B07	NM_001909.4	CTSD	Cathepsin D
B08	NM_001912.4 NM_145918.2 NM_001257973.1 NM_001257971.1 NM_001257972.1	CTSL1	Cathepsin L1
B09	NM_004079.4 NM_001199739.1	CTSS	Cathepsin S

B10	NM_005228.3 NM_201284.1 NM_201283.1 NM_201282.1	EGFR	Epidermal growth factor receptor
B11	NM_004448.2 NM_001005862.1	ERBB2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)
B12	NM_001982.3 NM_001005915.1	ERBB3	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
C01	NM_005235.2 NM_001042599.1	ERBB4	V-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)
C02	NM_000125.3 NM_001122742.1 NM_001122741.1 NM_001122740.1	ESR1	Estrogen receptor 1
C03	NM_001437.2 NM_001040276.1 NM_001040275.1 NM_001214903.1 NM_001214902.1	ESR2	Estrogen receptor 2 (ER beta)
C04	NM_004469.4	FIGF	C-fos induced growth factor (vascular endothelial growth factor D)
C05	NM_002019.4 NM_001160031.1 NM_001160030.1 NM_001159920.1	FLT1	Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)
C06	NM_002020.4 NM_182925.4	FLT4	Fms-related tyrosine kinase 4
C07	NM_002086.4 NM_203506.2	GRB2	Growth factor receptor-bound protein 2
C08	NM_000852.3	GSTP1	Glutathione S-transferase pi 1
C09	NM_004964.2	HDAC1	Histone deacetylase 1
C10	NM_024827.3 NM_001136041.2	HDAC11	Histone deacetylase 11
C11	NM_001527.3	HDAC2	Histone deacetylase 2
C12	NM_003883.3	HDAC3	Histone deacetylase 3
D01	NM_006037.3	HDAC4	Histone deacetylase 4
D02	NM_006044.2	HDAC6	Histone deacetylase 6
D03	NM_001098416.2 NM_015401.3	HDAC7	Histone deacetylase 7
D04	NM_018486.2 NM_001166420.1 NM_001166422.1 NM_001166419.1 NM_001166418.1 NM_001166448.1	HDAC8	Histone deacetylase 8
D05	NM_001530.3 NM_181054.2 NM_001243084.1	HIF1A	Hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
D06	NM_005343.2 NM_176795.3 NM_001130442.1	HRAS	V-Ha-ras Harvey rat sarcoma viral oncogene homolog
D07	NM_001017963.2 NM_005348.3	HSP90AA1	Heat shock protein 90kDa alpha (cytosolic), class A member 1
D08	NM_003299.2	HSP90B1	Heat shock protein 90kDa beta (Grp94), member 1
D09	NM_000618.3 NM_001111285.1 NM_001111284.1 NM_001111283.1	IGF1	Insulin-like growth factor 1 (somatomedin C)
D10	NM_000875.3	IGF1R	Insulin-like growth factor 1 receptor
D11	NM_000612.4 NM_000207.2	IGF2	Insulin-like growth factor 2 (somatomedin A)

	NM_001007139.4 NM_001042376.2 NM_001127598.1 NM_001185098.1 NM_001185097.1		
D12	NM_001098629.1 NM_001098627.2 NM_001098630.1 NM_032643.3 NM_001242452.1	IRF5	Interferon regulatory factor 5
E01	NM_002253.2	KDR	Kinase insert domain receptor (a type III receptor tyrosine kinase)
E02	NM_000222.2 NM_001093772.1	KIT	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
E03	NM_004985.3 NM_033360.2	KRAS	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
E04	NM_002392.4	MDM2	Mdm2 p53 binding protein homolog (mouse)
E05	NM_002393.4 NM_001204172.1 NM_001204171.1	MDM4	Mdm4 p53 binding protein homolog (mouse)
E06	NM_004958.3	MTOR	Mechanistic target of rapamycin (serine/threonine kinase)
E07	NM_003998.3 NM_001165412.1	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
E08	NM_002524.4	NRAS	Neuroblastoma RAS viral (v-ras) oncogene homolog
E09	NM_006181.2	NTN3	Netrin 3
E10	NM_001618.3	PARP1	Poly (ADP-ribose) polymerase 1
E11	NM_005484.3 NM_001042618.1	PARP2	Poly (ADP-ribose) polymerase 2
E12	NM_006437.3	PARP4	Poly (ADP-ribose) polymerase family, member 4
F01	NM_006206.4	PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide
F02	NM_002609.3	PDGFRB	Platelet-derived growth factor receptor, beta polypeptide
F03	NM_000926.4 NM_001202474.1	PGR	Progesterone receptor
F04	NM_002645.2	PIK3C2A	Phosphoinositide-3-kinase, class 2, alpha polypeptide
F05	NM_002647.2	PIK3C3	Phosphoinositide-3-kinase, class 3
F06	NM_006218.2	PIK3CA	Phosphoinositide-3-kinase, catalytic, alpha polypeptide
F07	NM_005030.3	PLK1	Polo-like kinase 1
F08	NM_006622.3 NM_001252226.1	PLK2	Polo-like kinase 2
F09	NM_004073.2	PLK3	Polo-like kinase 3
F10	NM_014264.4 NM_001190801.1 NM_001190799.1	PLK4	Polo-like kinase 4
F11	NM_002737.2	PRKCA	Protein kinase C, alpha
F12	NM_002738.6 NM_212535.2	PRKCB	Protein kinase C, beta
G01	NM_006254.3 NM_212539.1	PRKCD	Protein kinase C, delta
G02	NM_005400.2	PRKCE	Protein kinase C, epsilon
G03	NM_000963.2	PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
G04	NM_001664.2	RHOA	Ras homolog gene family, member A
G05	NM_004040.2	RHOB	Ras homolog gene family, member B
G06	NM_198253.2 NM_001193376.1	TERT	Telomerase reverse transcriptase
G07	NM_003747.2	TNKS	Tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase
G08	NM_001067.3	TOP2A	Topoisomerase (DNA) II alpha 170kDa
G09	NM_001068.2	TOP2B	Topoisomerase (DNA) II beta 180kDa
G10	NM_000546.5 NM_001126117.1	TP53	Tumor protein p53

	NM_001126116.1 NM_001126115.1 NM_001126114.2 NM_001126113.2 NM_001126112.2 NM_001126118.1		
G11	NM_003329.3 NM_001244938.1	TXN	Thioredoxin
G12	NM_003330.3 NM_182743.2 NM_182742.2 NM_182729.2 NM_001093771.2 NM_001261446.1 NM_001261445.1	TXNRD1	Thioredoxin reductase 1
H1	NM_005427.3 NM_001126242.2 NM_001126241.2 NM_001126240.2 NM_001204192.1 NM_001204191.1 NM_001204190.1 NM_001204188.1 NM_001204185.1 NM_001204187.1 NM_001204184.1 NM_001204189.1 NM_001204186.1	TP73	Tumor protein p73
H2	NM_003403.3	YY1	YY1 transcription factor
H3	NM_012423.3 NM_001270491.1	RPL13A	Ribosomal protein L13a
H4	NM_004048.2	B2M	Beta-2-microglobulin
H5	BSG-PAH01	HGD1	Genomic DNA control
H6	BSG-PAH02	HGD	Genomic DNA control
H7	NM_002046.4 NM_001256799.1	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H8	NM_002046.4 NM_001256799.1	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H9	NM_001101.3	ACTB	Actin, beta
H10	NM_001101.3	ACTB	Actin, beta
H11	NM_006082.2	tuba1b	Homo sapiens tubulin, alpha 1b (TUBA1B)
H12	NM_000194.2	HPRT1	Hypoxanthine phosphoribosyltransferase 1