Human TLR Pathways qPCR Array

Catalogue # GA-R204A, GA-R204B

Description

Toll-like receptors (TLRs) are single, membrane-spanning, non-catalytic receptors that activate immune cell responses upon microbes invasion. This Human TLR Pathways Array is designed to profile the expression of **88 genes for the receptors in the TLR family as well as the adapter proteins and kinases that mediate TLR signaling.**

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. A few additional house-keeping genes are used as positive controls.

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™ 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)

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
BTK	*CASP8*	*CCL2*	*CD14*	*CD80*	*CD86*	*CHUK*	*CLEC4E*	*CSF2*	*CSF3*	*CXCL10*	*EIF2AK2*
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
ELK1	*FADD*	*FOS*	*HMGB1*	*HRAS*	*HSPA1A*	*HSPD1*	*IFNA1*	*IFNB1*	*IFNG*	*IKBKB*	*IL10*
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
IL12A	*IL1A*	*IL1B*	*IL2*	*IL6*	*IL8*	*IRAK1*	*IRAK2*	*IRF1*	*IRF3*	*JUN*	*LTA*
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
CD180	*LY86*	*LY96*	*MAP2K3*	*MAP2K4*	*MAP3K1*	*MAP3K7*	*TAB1*	*MAP4K4*	*MAPK8*	*MAPK8IP3*	*MYD88*
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
NFKB1	*NFKB2*	*NFKBIA*	*NFKBIL1*	*NFRKB*	*NR2C2*	*PELI1*	*PPARA*	*PRKRA*	*PTGS2*	*REL*	*RELA*
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
RIPK2	*SARM1*	*SIGIRR*	*ECSIT*	*TBK1*	*TICAM2*	*TIRAP*	*TLR1*	*TLR10*	*TLR2*	*TLR3*	*TLR4*
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
TLR5	*TLR6*	*TLR7*	*TLR8*	*TLR9*	*TNF*	*TNFRSF1A*	*TOLLIP*	*TRAF6*	*TICAM1*	*UBE2N*	*UBE2V1*
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
RIPK3	*RGS2*	*RPL13A*	*B2M*	*HGD1*	*HGD2*	*GAPDH*	*GAPDH*	*ACTB*	*ACTB*	*TUBA1B*	*HPRT1*

Tel: 1-240-683-5851 • Fax: 1-240-683-5852 • info@eEnzyme.com • www.eEnzyme.com

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 TLR Pathways qPCR Array trial size (***Cat# GA-R204A1***)	Human TLR Pathways qPCR Array (***Cat# GA-R204A***)
96-Well Plate Containing Dried Assays ***(Part# R204-120)***	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 TLR Pathways qPCR Array trial size (***Cat# GA-R204B1***)	Human TLR Pathways qPCR Array (***Cat# GA-R204B***)
96-Well Plate Containing Dried Assays ***(Part# R204-120)***	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.

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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@eenzyme.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.
   1.1 Carefully remove the PCR Array from its sealed bag.
   1.2 Dispense Experimental Cocktail to PCR Array Loading Reservoir to assist in loading (optional).
   1.3 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.

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
A1	NM_000061.2	BTK	Bruton agammaglobulinemia tyrosine kinase
A2	NM_001228.4 NM_001080125.1 NM_001080124.1 NM_033358.3 NM_033356.3 NM_033355.3	CASP8	Caspase 8, apoptosis-related cysteine peptidase
A3	NM_002982.3	CCL2	Chemokine (C-C motif) ligand 2
A4	NM_000591.3 NM_001040021.2 NM_001174105.1 NM_001174104.1	CD14	CD14 molecule
A5	NM_005191.3	CD80	CD80 molecule
A6	NM_006889.4 NM_175862.4 NM_001206925.1 NM_001206924.1 NM_176892.1	CD86	CD86 molecule
A7	NM_001278.3	CHUK	Conserved helix-loop-helix ubiquitous kinase
A8	NM_014358.2	CLEC4E	C-type lectin domain family 4, member E
A9	NM_000758.3	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)
A10	NM_000759.3 NM_001178147.1 NM_172220.2 NM_172219.2	CSF3	Colony stimulating factor 3 (granulocyte)
A11	NM_001565.3	CXCL10	Chemokine (C-X-C motif) ligand 10
A12	NM_002759.3 NM_001135652.2 NM_001135651.2	EIF2AK2	Eukaryotic translation initiation factor 2-alpha kinase 2
B1	NM_005229.4 NM_001114123.2 NM_001257168.1	ELK1	ELK1, member of ETS oncogene family
B2	NM_003824.3	FADD	Fas (TNFRSF6)-associated via death domain
B3	NM_005252.3	FOS	FBJ murine osteosarcoma viral oncogene homolog
B4	NM_002128.4	HMGB1	High mobility group box 1
B5	NM_005343.2 NM_176795.3 NM_001130442.1	HRAS	V-Ha-ras Harvey rat sarcoma viral oncogene homolog
B6	NM_005345.5	HSPA1A	Heat shock 70kDa protein 1A
B7	NM_002156.4 NM_199440.1	HSPD1	Heat shock 60kDa protein 1 (chaperonin)
B8	NM_024013.2	IFNA1	Interferon, alpha 1
B9	NM_002176.2	IFNB1	Interferon, beta 1, fibroblast
B10	NM_000619.2	IFNG	Interferon, gamma
B11	NM_001556.2 NM_001190720.2 NM_001242778.1	IKBKB	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
B12	NM_000572.2	IL10	Interleukin 10
C1	NM_000882.3	IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)
C2	NM_000575.3	IL1A	Interleukin 1, alpha
C3	NM_000576.2	IL1B	Interleukin 1, beta
C4	NM_000586.3	IL2	Interleukin 2

C5	NM_000600.3	IL6	Interleukin 6 (interferon, beta 2)
C6	NM_000584.3	IL8	Interleukin 8
C7	NM_001569.3 NM_001025243.1 NM_001025242.1	IRAK1	Interleukin-1 receptor-associated kinase 1
C8	NM_001570.3	IRAK2	Interleukin-1 receptor-associated kinase 2
C9	NM_002198.2	IRF1	Interferon regulatory factor 1
C10	NM_001571.5 NM_001197128.1 NM_001197127.1 NM_001197125.1 NM_001197126.1 NM_001197124.1 NM_001197123.1 NM_001197122.1	IRF3	Interferon regulatory factor 3
C11	NM_002228.3	JUN	Jun proto-oncogene
C12	NM_000595.3 NM_001159740.2	LTA	Lymphotoxin alpha (TNF superfamily, member 1)
D1	NM_005582.2	CD180	CD180 molecule
D2	NM_004271.3	LY86	Lymphocyte antigen 86
D3	NM_015364.4 NM_001195797.1	LY96	Lymphocyte antigen 96
D4	NM_002756.4 NM_145109.2	MAP2K3	Mitogen-activated protein kinase kinase 3
D5	NM_003010.2	MAP2K4	Mitogen-activated protein kinase kinase 4
D6	NM_005921.1	MAP3K1	Mitogen-activated protein kinase kinase kinase 1, E3 ubiquitin protein ligase
D7	NM_003188.3 NM_145333.2 NM_145332.2 NM_145331.2	MAP3K7	Mitogen-activated protein kinase kinase kinase 7
D8	NM_006116.2 NM_153497.2	TAB1	TGF-beta activated kinase 1/MAP3K7 binding protein 1
D9	NM_004834.4 NM_145687.3 NM_145686.3 NM_001242560.1 NM_001242559.1	MAP4K4	Mitogen-activated protein kinase kinase kinase kinase 4
D10	NM_002750.2 NM_139049.1 NM_139047.1 NM_139046.1	MAPK8	Mitogen-activated protein kinase 8
D11	NM_015133.3 NM_001040439.1	MAPK8IP3	Mitogen-activated protein kinase 8 interacting protein 3
D12	NM_002468.4 NM_001172568.1 NM_001172569.1 NM_001172567.1 NM_001172566.1	MYD88	Myeloid differentiation primary response gene (88)
E1	NM_003998.3 NM_001165412.1	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
E2	NM_002502.4 NM_001077494.2 NM_001261403.1	NFKB2	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)
E3	NM_020529.2	NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
E4	NM_005007.3 NM_001144963.1 NM_001144962.1 NM_001144961.1	NFKBIL1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1
E5	NM_006165.3 NM_001143835.1	NFRKB	Nuclear factor related to kappaB binding protein

E6	NM_003298.3	NR2C2	Nuclear receptor subfamily 2, group C, member 2
E7	NM_020651.3	PELI1	Pellino E3 ubiquitin protein ligase 1
E8	NM_005036.4 NM_001001928.2	PPARA	Peroxisome proliferator-activated receptor alpha
E9	NM_003690.4 NM_001139518.1 NM_001139517.1	PRKRA	Protein kinase, interferon-inducible double stranded RNA dependent activator
E10	NM_000963.2	PTGS2	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
E11	NM_002908.2	REL	V-rel reticuloendotheliosis viral oncogene homolog (avian)
E12	NM_021975.3 NM_001145138.1 NM_001243985.1 NM_001243984.1	RELA	V-rel reticuloendotheliosis viral oncogene homolog A (avian)
F1	NM_003821.5	RIPK2	Receptor-interacting serine-threonine kinase 2
F2	NM_015077.2	SARM1	Sterile alpha and TIR motif containing 1
F3	NM_021805.2 NM_001135054.1 NM_001135053.1	SIGIRR	Single immunoglobulin and toll-interleukin 1 receptor (TIR) domain
F4	NM_016581.4 NM_001142465.2 NM_001142464.2 NM_001243204.1	ECSIT	ECSIT homolog (Drosophila)
F5	NM_013254.3	TBK1	TANK-binding kinase 1
F6	NM_021649.6 NM_181836.5 NM_001164469.2 NM_001164468.2	TICAM2	Toll-like receptor adaptor molecule 2
F7	NM_001039661.1 NM_148910.2	TIRAP	Toll-interleukin 1 receptor (TIR) domain containing adaptor protein
F8 *(F8)*	NM_003263.3	TLR1	Toll-like receptor 1
F9	NM_030956.3 NM_001017388.2 NM_001195108.1 NM_001195107.1 NM_001195106.1	TLR10	Toll-like receptor 10
F10	NM_003264.3	TLR2	Toll-like receptor 2
F11	NM_003265.2	TLR3	Toll-like receptor 3
F12	NM_138554.4 NM_003266.3 NM_138557.2	TLR4	Toll-like receptor 4
G1	NM_003268.5	TLR5	Toll-like receptor 5
G2	NM_006068.4	TLR6	Toll-like receptor 6
G3	NM_016562.3	TLR7	Toll-like receptor 7
G4	NM_138636.4	TLR8	Toll-like receptor 8
G5	NM_017442.3	TLR9	Toll-like receptor 9
G6	NM_000594.3	TNF	Tumor necrosis factor
G7	NM_001065.3	TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A
G8	NM_019009.3	TOLLIP	Toll interacting protein
G9	NM_004620.3 NM_145803.2	TRAF6	TNF receptor-associated factor 6, E3 ubiquitin protein ligase
G10	NM_182919.3	TICAM1	Toll-like receptor adaptor molecule 1
G11	NM_003348.3	UBE2N	Ubiquitin-conjugating enzyme E2N
G12	NM_021988.5 NM_199129.2 NM_199203.2 NM_199144.2	UBE2V1	Ubiquitin-conjugating enzyme E2 variant 1

	NM_001032288.2 NM_022442.5 NM_001162505.1 NM_001257395.1 NM_001257394.1 NM_001257393.1 NM_001257399.1 NM_001257398.1 NM_001257397.1 NM_001257396.1		
H1	NM_006871.3	RIPK3	Receptor-interacting serine-threonine kinase 3
H2	NM_002923.3	RGS2	Regulator of G-protein signaling 2, 24kDa
H3	NM_012423.3 NM_001270491.1	RPL13A	Ribosomal protein L13a
H4	NM_004048.2	B2M	Beta-2-microglobulin
H5	BSG-0001	HGD1	Human Genomic DNA Contamination
H6	BSG-0002	HGD2	Human Genomic DNA Contamination
H7	NM_002046.4 NM_001256799.1	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H8	NM_002046.4 NM_001256799.1	GAPDH(1)	Glyceraldehyde-3-phosphate dehydrogenase
H9	NM_001101.3	ACTB	Actin, beta
H10	NM_001101.3	ACTB(1)	Actin, beta
H11	NM_006082.2	Tuba1b	Homo sapiens tubulin, alpha 1b (TUBA1B)
H12	NM_000194.2	HPRT1	Hypoxanthine phosphoribosyltransferase 1