Human Athma-Allergy qPCR Array

Catalogue # GA-A115A, GA-A115B

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 Asthma-Allergy Array is designed to profile the expression of **88 key genes involved in allergic responses** with additional house-keeping genes 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™ 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)

A1 *ADAM33*	A2 *ADRB2*	A3 *ALOX5*	A4 *ARG1*	A5 *BCL6*	A6 *CCL11*	A7 *CCL17*	A8 *CCL2*	A9 *CCL22*	A10 *CCL24*	A11 *CCL26*	A12 *CCL5*
B1 *CCL8*	B2 *CCR3*	B3 *CCR4*	B4 *CCR8*	B5 *CD40LG*	B6 *CHI3L1*	B7 *CHIA*	B8 *CLC*	B9 *CLCA1*	B10 *CMA1*	B11 *CPA3*	B12 *CRLF2*
C1 *CSF2*	C2 *CSF3R*	C3 *CYSLTR1*	C4 *EPX*	C5 *FCER1A*	C6 *FOXP3*	C7 *GATA3*	C8 *GPR44*	C9 *ICOS*	C10 *IFNG*	C11 *IFNGR2*	C12 *IL10*
D1 *IL12A*	D2 *IL12B*	D3 *IL13*	D4 *IL13RA1*	D5 *IL13RA2*	D6 *IL17A*	D7 *IL17RB*	D8 *IL18*	D9 *IL1RL1*	D10 *IL21*	D11 *IL25*	D12 *IL2RA*
E1 *IL3*	E2 *IL31*	E3 *IL33*	E4 *IL3RA*	E5 *IL4*	E6 *IL4R*	E7 *IL5*	E8 *IL5RA*	E9 *IL9*	E10 *KIT*	E11 *KITLG*	E12 *LTB4R*
F1 *MAF*	F2 *MMP9*	F3 *MRC1*	F4 *MS4A2*	F5 *MUC5AC*	F6 *PDCD1*	F7 *PMCH*	F8 *POSTN*	F9 *PPARG*	F10 *PRG2*	F11 *RETNLB*	F12 *RNASE2*
G1 *RNASE3*	G2 *RORC*	G3 *SATB1*	G4 *SIGLEC8*	G5 *STAT5A*	G6 *STAT6*	G7 *TBX21*	G8 *TGFB1*	G9 *TNFRSF4*	G10 *TNFSF4*	G11 *TPSAB1*	G12 *TSLP*
H1 *B2M*	H2 *MMP1*	H3 *RPL13A*	H4 *MMP3*	H5 *HGD1*	H6 *HGD2*	H7 *GAPDH*	H8 *GAPDH*	H9 *ACTB*	H10 *ACTB*	H11 *TUBA1B*	H12 *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 Athma-Allergy qPCR Array trial size (***Cat# GA-A115A1***)	Human Athma-Allergy qPCR Array (***Cat# GA-A115A***)
96-Well Plate Containing Dried Assays ***(Part# A115-120)***	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 Athma-Allergy qPCR Array trial size (***Cat# GA-A115B1***)	Human Atham-Allergy qPCR Array (***Cat# GA-A115B***)
96-Well Plate Containing Dried Assays ***(Part# A115-120)***	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_025220.2 NM_153202.1	ADAM33	ADAM metallopeptidase domain 33
A2	NM_000024.5	ADRB2	Adrenergic, beta-2-, receptor, surface
A3	NM_000698.3 NM_001256154.1 NM_001256153.1	ALOX5	Arachidonate 5-lipoxygenase
A4	NM_000045.3 NM_001244438.1	ARG1	Arginase, liver
A5	NM_001706.4 NM_001130845.1 NM_001134738.1	BCL6	B-cell CLL/lymphoma 6
A6	NM_002986.2	CCL11	Chemokine (C-C motif) ligand 11
A7	NM_002987.2	CCL17	Chemokine (C-C motif) ligand 17
A8	NM_002982.3	CCL2	Chemokine (C-C motif) ligand 2
A9	NM_002990.4	CCL22	Chemokine (C-C motif) ligand 22
A10	NM_002991.2	CCL24	Chemokine (C-C motif) ligand 24
A11	NM_006072.4	CCL26	Chemokine (C-C motif) ligand 26
A12	NM_002985.2	CCL5	Chemokine (C-C motif) ligand 5
B1	NM_005623.2	CCL8	Chemokine (C-C motif) ligand 8
B2	NM_001837.3 NM_178329.2 NM_001164680.1 NM_178328.1	CCR3	Chemokine (C-C motif) receptor 3
B3	NM_005508.4	CCR4	Chemokine (C-C motif) receptor 4
B4	NM_005201.3	CCR8	Chemokine (C-C motif) receptor 8
B5	NM_000074.2	CD40LG	CD40 ligand
B6	NM_001276.2	CHI3L1	Chitinase 3-like 1 (cartilage glycoprotein-39)
B7	NM_201653.3 NM_021797.3 NM_001040623.2 NM_001258004.1 NM_001258003.1 NM_001258001.1 NM_001258005.1 NM_001258002.1	CHIA	Chitinase, acidic
B8	NM_001828.5	CLC	Charcot-Leyden crystal protein
B9	NM_001285.3	CLCA1	Chloride channel accessory 1
B10	NM_001836.3	CMA1	Chymase 1, mast cell
B11	NM_001870.3	CPA3	Carboxypeptidase A3 (mast cell)
B12	NM_001012288.1 NM_022148.2	CRLF2	Cytokine receptor-like factor 2
C1	NM_000758.3	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)
C2	NM_000760.3 NM_156039.3 NM_172313.2	CSF3R	Colony stimulating factor 3 receptor (granulocyte)
C3	NM_006639.2	CYSLTR1	Cysteinyl leukotriene receptor 1
C4	NM_000502.4	EPX	Eosinophil peroxidase
C5	NM_002001.3	FCER1A	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide
C6	NM_014009.3 NM_001114377.1	FOXP3	Forkhead box P3
C7	NM_002051.2 NM_001002295.1	GATA3	GATA binding protein 3

C8	NM_004778.2	GPR44	G protein-coupled receptor 44
C9	NM_012092.3	ICOS	Inducible T-cell co-stimulator
C10	NM_000619.2	IFNG	Interferon, gamma
C11	NM_005534.3	IFNGR2	Interferon gamma receptor 2 (interferon gamma transducer 1)
C12	NM_000572.2	IL10	Interleukin 10
D1	NM_000882.3	IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)
D2	NM_002187.2	IL12B	Interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)
D3	NM_002188.2	IL13	Interleukin 13
D4	NM_001560.2	IL13RA1	Interleukin 13 receptor, alpha 1
D5	NM_000640.2	IL13RA2	Interleukin 13 receptor, alpha 2
D6	NM_002190.2	IL17A	Interleukin 17A
D7	NM_018725.3	IL17RB	Interleukin 17 receptor B
D8	NM_001562.3 NM_001243211.1	IL18	Interleukin 18 (interferon-gamma-inducing factor)
D9	NM_016232.4	IL1RL1	Interleukin 1 receptor-like 1
D10	NM_021803.3 NM_001207006.2	IL21	Interleukin 21
D11	NM_022789.3 NM_172314.1	IL25	Interleukin 25
D12	NM_000417.2	IL2RA	Interleukin 2 receptor, alpha
E1	NM_000588.3	IL3	Interleukin 3 (colony-stimulating factor, multiple)
E2	NM_001014336.1	IL31	Interleukin 31
E3	NM_033439.3 NM_001199641.1 NM_001199640.1	IL33	Interleukin 33
E4	NM_002183.3 NM_001267713.1	IL3RA	Interleukin 3 receptor, alpha (low affinity)
E5	NM_000589.3 NM_172348.2	IL4	Interleukin 4
E6	NM_000418.3 NM_001257997.1 NM_001257406.1 NM_001257407.1	IL4R	Interleukin 4 receptor
E7	NM_000879.2	IL5	Interleukin 5 (colony-stimulating factor, eosinophil)
E8	NM_000564.4 NM_175728.2 NM_175727.2 NM_175726.3 NM_175725.2 NM_175724.2 NM_001243099.1	IL5RA	Interleukin 5 receptor, alpha
E9	NM_000590.1	IL9	Interleukin 9
E10	NM_000222.2 NM_001093772.1	KIT	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
E11	NM_003994.5 NM_000899.4	KITLG	KIT ligand
E12	NM_181657.3 NM_001143919.2	LTB4R	Leukotriene B4 receptor
F1	NM_005360.4	MAF	V-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)
F2	NM_004994.2	MMP9	Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
F3	NM_002438.2	MRC1	Mannose receptor, C type 1
F4	NM_000139.4 NM_001256916.1	MS4A2	Membrane-spanning 4-domains, subfamily A, member 2 (Fc fragment of IgE, high affinity I, receptor for; beta polypeptide)
F5	XM_003403450	MUC5AC	Mucin 5AC, oligomeric mucus/gel-forming
F6	NM_005018.2	PDCD1	Programmed cell death 1

F7	NM_002674.2	PMCH	Pro-melanin-concentrating hormone
F8 *(F8)*	NM_006475.2 NM_001135936.1 NM_001135935.1 NM_001135934.1	POSTN	Periostin, osteoblast specific factor
F9	NM_015869	PPARG	Peroxisome proliferator-activated receptor gamma
F10	NM_002728	PRG2	Proteoglycan 2, bone marrow (natural killer cell activator, eosinophil granule major basic protein)
F11	NM_032579	RETNLB	Resistin like beta
F12	NM_002934	RNASE2	Ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin)
G1	NM_002935	RNASE3	Ribonuclease, RNase A family, 3
G2	NM_005060	RORC	RAR-related orphan receptor C
G3	NM_002971	SATB1	SATB homeobox 1
G4	NM_014442	SIGLEC8	Sialic acid binding Ig-like lectin 8
G5	NM_003152	STAT5A	Signal transducer and activator of transcription 5A
G6	NM_003153.4 NM_001178081.1 NM_001178080.1 NM_001178079.1 NM_001178078.1	STAT6	Signal transducer and activator of transcription 6, interleukin-4 induced
G7	NM_013351.1	TBX21	T-box 21
G8	NM_000660.4	TGFB1	Transforming growth factor, beta 1
G9	NM_003327.3	TNFRSF4	Tumor necrosis factor receptor superfamily, member 4
G10	NM_003326.3	TNFSF4	Tumor necrosis factor (ligand) superfamily, member 4
G11	NM_003294.3	TPSAB1	Tryptase alpha/beta 1
G12	NM_033035.4	TSLP	Thymic stromal lymphopoietin
H1	NM_004048.2	B2M	Beta-2-microglobulin
H2	NM_002421.3 NM_001145938.1	MMP1	Matrix metallopeptidase 1 (interstitial collagenase)
H3	NM_012423.3 NM_001270491.1	RPL13A	Ribosomal protein L13a
H4	NM_002422.3	MMP3	Matrix metallopeptidase 3 (stromelysin 1, progelatinase)
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	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