GeneQuery™ Human Inflammatory Chemokines, Interleukins, and Receptors qPCR Array Kit (GQH-CHE)

Catalog #GK110

Product Description

ScienCell's GeneQuery™ Human Inflammatory Chemokines, Interleukins, and Receptors qPCR Array Kit (GQH-CHE) surveys 88 key genes involved in first steps of inflammation signaling. Inflammation occurs as a protective response against harmful stimuli and as a signal to initiate tissue repair. The initiation of inflammation involves immune cell migration to the insulted site by chemotaxis and immune cell activation by cytokines. This kit focuses on chemokines and interleukins involved in the initiation of inflammation. Note: For a survey of 88 additional cytokines involved in the initial inflammatory response, a separate GeneQuery array is available (GK109; Human Inflammatory Cytokines and Receptors). Brief examples of how included genes may be categorized are shown below:

- **Chemokines:** CCL1, CCL20, CCL5, CX3CL1, CXCL9
- **Chemokine receptors:** CCR1, CCR5, CCR9, CX3CR1, CXCL2
- **Interleukins:** IL10, IL2, IL6, IL12A, IL9
- **Interleukin receptors:** IL10RA, IL2RA, IL6R, IL12RB1, IL9R

GeneQuery™ qPCR array kits are qPCR ready in a 96-well plate format, with each well containing one primer set that recognizes and efficiently amplifies a specific target gene's cDNA. The carefully designed primers ensure that: (i) the optimal annealing temperature in qPCR analysis is 65°C (with 2 mM Mg^{2+} and no DMSO); (ii) the primer set recognizes all known transcript variants of the target gene, unless otherwise noted; and (iii) only one gene is amplified. Each primer set has been validated by qPCR with melt curve analysis and gel electrophoresis.

GeneQuery™ qPCR Array Kit Controls

Each GeneQuery™ plate contains eight controls (Figure 1):

- Five target housekeeping genes (β-actin, GAPDH, LDHA, NONO, and PPIH), which enable normalization of data.
- The Genomic DNA (gDNA) Control (GDC), which detects gDNA contamination in cDNA samples. This primer set targets a non-transcribed region of the genome.
- Positive PCR Control (PPC), which tests whether samples contain inhibitors or other factors that may negatively affect gene expression results. The PPC consists of a predispensed synthetic DNA template and a primer set that can amplify it. The sequence of the DNA template is not present in the human genome and thus tests the efficiency of the polymerase chain reaction itself.
- The No Template Control (NTC), which can be used to monitor DNA contamination introduced during workflow (e.g. from such sources as reagents, tips, and the lab bench).

Kit Components

Component	Quantity	Storage
GeneQuery™ array plate with lyophilized primers	1	4°C or -20°C
Optical PCR plate seal	1	RT
Nuclease-free H_2O	2 mL	4°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
Reverse transcriptase	MultiScribe Reverse Transcriptase (Life Tech, Cat. #4311235)
cDNA template	Customers' samples
qPCR master mix	FastStart Essential DNA Green Master (Roche, Cat. #06402712001)

Quality Control

All primer sets are validated by qPCR with melt curve analysis and analyzed by gel electrophoresis. Single band amplification is confirmed for each set of primers.

Product Use

GQH-ANG is for research use only. It is not approved for human or animal use or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

This product is shipped at ambient temperature. Upon receipt, the plate should be stored at 4°C and is good for up to 12 months. For long-term storage (>1 year), store at -20°C in a manual defrost freezer.

Procedures

Note: The primers in each well are lyophilized.

1. Prior to use, allow plates to warm to room temperature.
2. Briefly centrifuge at 1,500x g for 1 minute before slowly peeling off the seal.
3. Prepare 20 µl PCR reactions for one well as shown in Table 1.

Table 1

cDNA template	**0.2 – 250 ng**
2x qPCR master mix	10 µl
Nuclease-free H_2O	variable
Total volume	***20 µl***

***Important: Only** use polymerases with hot-start capability to prevent possible primer-dimer formation. **Only** use nuclease-free reagents in PCR* amplification.

4. Add the mixture of 2x qPCR master mix, cDNA template, and nuclease-free H_2O to each well containing the lyophilized primers. Seal the plate with the provided optical PCR plate seal.

Important: In NTC control well, do NOT add cDNA template. Add 2x qPCR master mix and nuclease-free H2O only.

5. Briefly centrifuge the plates at 1,500x g for 1 minute at room temperature. For maximum reliability, replicates are strongly recommended (minimum of 3).

6. For PCR program setup, please refer to the instructions of the master mix of the user's choice. We recommend a typical 3-step qPCR protocol for a 200nt amplicon:

Three-step cycling protocol

Step	**Temperature**	**Time**	**Number of cycles**
Initial denaturation	95°C	10 min	1
Denaturation	95°C	20 sec	40
Annealing	65°C	20 sec	
Extension	72°C	20 sec	
Data acquisition	Plate read		
Recommended	*Melting curve analysis*		1
Hold	4°C	Indefinite	1

7. (Optional) Load the PCR products on 1.5% agarose gel and perform electrophoresis to confirm the single band amplification in each well.

Figure 1. Layout of GeneQuery™ qPCR array kit controls.

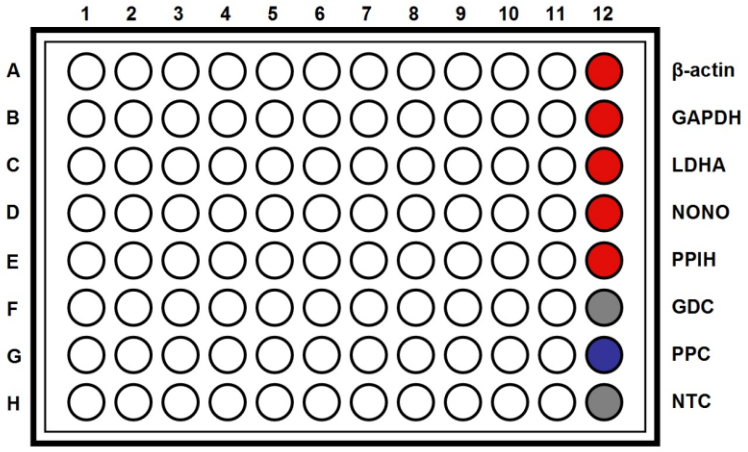


Table 2. Interpretation of control results:

Controls	***Results***	***Interpretation***	***Suggestions***
Housekeeping gene controls	Variability of a housekeeping gene's Cq value	The expression of the housekeeping gene is variable in samples; cycling program is incorrect	Choose a constantly expressed target, or analyze expression levels of multiple housekeeping genes; use correct cycling program and make sure that all cycle parameters have been correctly entered
gDNA Control (GDC)	Cq ≥ 35	No gDNA detected	N/A
	Cq < 35	The sample is contaminated with gDNA	Perform DNase digestion during RNA purification step
Positive PCR Control (PPC)	Cq > 30; or The Cq variations > 2 between qPCR Arrays.	Poor PCR performance; possible PCR inhibitor in reactions; cycling program incorrect	Eliminate inhibitor by purifying samples; use correct cycling program and make sure that all cycle parameters have been correctly entered
No Template Control (NTC)	Positive	DNA contamination in workflow	Eliminate sources of DNA contamination (reagents, plastics, etc.)

Figure 2. A typical amplification curve showing the amplification of a qPCR product.

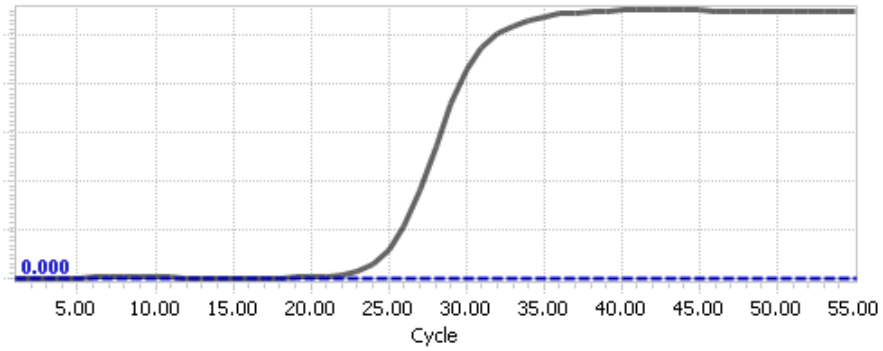
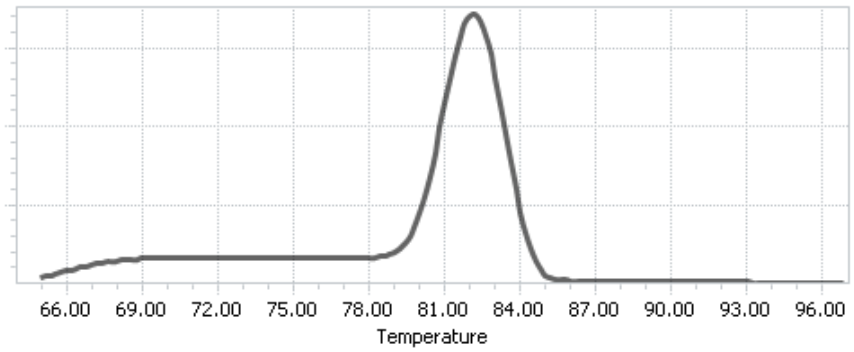


Figure 3. A typical melting peak of a qPCR product.



Quantification Method: Comparative ΔΔCq (Quantification Cycle Value) Method

1. **Note:** Please refer to your qPCR instrument's data analysis software for data analysis. The method provided here serves as guidance for quick manual calculations.

   You can use one or more housekeeping genes as a reference to normalize samples.

   Important: We highly recommend using all 5 housekeeping genes included in this kit, β-actin, GAPDH, LDHA, NONO, and PPIH.

2. For a single housekeeping gene, ΔCq (ref) is the quantification cycle number change for that housekeeping gene (HKG) between an experimental sample and control sample.

   $$\Delta Cq\ (ref) = Cq\ (HKG, experimental\ sample) - Cq\ (HKG, control\ sample)$$

   When using multiple housekeeping genes as a reference, we recommend normalizing using the geometric mean [1] of the expression level change, which is the same as normalizing using the arithmetic mean of ΔCq of the selected housekeeping genes.

   $$\Delta Cq\ (ref) = average\ (\Delta Cq\ (HKG1), \Delta Cq\ (HKG2), ..., \Delta Cq\ (HKG\ n))$$ (n is the number of housekeeping genes selected)

   If using all 5 housekeeping genes included in this kit, β-actin, GAPDH, LDHA, NONO, and PPIH, use the following formula:

   $$\Delta Cq\ (ref) = (\Delta Cq(\beta\text{-actin}) + \Delta Cq(GAPDH) + \Delta Cq(LDHA) + \Delta Cq(NONO) + \Delta Cq(PPIH)) / 5$$

   Note: ΔCq (HKG) = Cq (HKG, experimental sample) - Cq (HKG, control sample), and ΔCq (HKG) value can be positive, 0, or negative.

3. For any of your genes of interest (GOI),

   $$\Delta Cq\ (GOI) = Cq\ (GOI, experimental\ sample) - Cq\ (GOI, control\ sample)$$

   $$\Delta\Delta Cq = \Delta Cq\ (GOI) - \Delta Cq\ (ref)$$

   $$Normalized\ GOI\ expression\ level\ fold\ change = 2^{-\Delta\Delta Cq}$$

References

[1] Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. (2002) "Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes." *Genome Biol.* 3(7): 1-12.

Example: Comparative ΔΔCq (Quantification Cycle Value) Method

Table 3. Cq (Quantification Cycle) values of 2 genes-of-interest and 5 housekeeping genes obtained for experimental and control samples.

	Genes of Interest		Housekeeping Genes				
Samples	**GOI1**	**GOI2**	***β-actin***	***GAPDH***	***LDHA***	***NONO***	***PPIH***
Experimental	21.61	22.19	17.16	17.84	20.12	19.64	26.40
Control	33.13	26.47	18.20	18.48	20.57	19.50	26.55

$\Delta Cq\ (ref) = (\Delta Cq(\beta\text{-actin})+\Delta Cq(GAPDH)+\Delta Cq(LDHA)+\Delta Cq(NONO)+\Delta Cq(PPIH))\ /5$
$= ((17.16-18.20)+(17.84-18.48)+(20.12-20.57)+(19.64-19.50)+(26.40-26.55))/5$
$= -0.43$

$\Delta Cq\ (GOI1) = 21.61 - 33.13$
$= -11.52$

$\Delta Cq\ (GOI2) = 22.19 - 26.47$
$= -4.28$

$\Delta\Delta Cq\ (GOI1) = \Delta Cq\ (GOI1) - \Delta Cq\ (ref)$
$= -11.52 - (-0.43)$
$= -11.09$

$\Delta\Delta Cq\ (GOI2) = \Delta Cq\ (GOI2) - \Delta Cq\ (ref)$
$= -4.28 - (-0.43)$
$= -3.85$

Normalized GOI1 expression level fold change $= 2^{-\Delta\Delta Cq\ (GOI1)}$
$= 2^{11.09}$
$= 2180$

Normalized GOI2 expression level fold change $= 2^{-\Delta\Delta Cq\ (GOI2)}$
$= 2^{3.85}$
$= 14.4$

Conclusion: Upon treatment, expression level of GOI1 increased 2,180 fold, and expression level of GOI2 increased 14.4 fold.

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GeneQuery™ Inflammatory Chemokines, Interleukins, and Receptors qPCR Array Plate Layout* (***8 controls*** in Bold and Italic)

	1	2	3	4	5	6	7	8	9	10	11	12
A	CCL1	CCL20	CCR1	CCR9	CXCL2	CXCR5	IL12RB1	IL17A	IL1R1	IL2RB	IL5	***β-actin***
B	CCL11	CCL22	CCR2	CCRL2	CXCL3	CXCR6	IL12RB2	IL17B	IL1R2	IL2RG	IL5RA	***GAPDH***
C	CCL14	CCL24	CCR3	CX3CL1	CXCL8	IL10	IL13	IL17C	IL2	IL3	IL6	***LDHA***
D	CCL15	CCL27	CCR4	CX3CR1	CXCL9	IL10RA	IL13RA1	IL17RA	IL20	IL31RA	IL6R	***NONO***
E	CCL17	CCL3	CCR5	CXCL1	CXCR1	IL10RB	IL13RA2	IL18	IL21R	IL32	IL7	***PPIH***
F	CCL18	CCL4	CCR6	CXCL10	CXCR2	IL11	IL15	IL18R1	IL23A	IL3RA	IL7R	***GDC***
G	CCL19	CCL5	CCR7	CXCL11	CXCR3	IL12A	IL15RA	IL1A	IL27RA	IL4	IL9	***PPC***
H	CCL2	CCL8	CCR8	CXCL12	CXCR4	IL12B	IL16	IL1B	IL2RA	IL4R	IL9R	***NTC***

* gene selection may be updated based on new research and development

Appendix. Plate type choice chart.

Plate type A

Brand	Model	kit catalog #
ABI / Life Tech	ABI 5700	GK110-A
	ABI 7000	GK110-A
	ABI 7300	GK110-A
	ABI 7500	GK110-A
	ABI 7700	GK110-A
	ABI 7900 HT	GK110-A
	QuantStudio	GK110-A
	ViiA 7	GK110-A
Bio-Rad	Chromo4	GK110-A
	iCycler	GK110-A
	iQ5	GK110-A
	MyiQ	GK110-A
	MyiQ2	GK110-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK110-A
	Matercycler ep realplex 4	GK110-A
Stratagene	MX3000P	GK110-A
	MX3005P	GK110-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK110-B
	ABI 7900 HT Fast	GK110-B
	QuantStudio Fast	GK110-B
	StepOnePlus	GK110-B
	ViiA 7 Fast	GK110-B
Bio-Rad	CFX Connect	GK110-B
	CFX96	GK110-B
	DNA Engine Opticon 2	GK110-B
Stratagene	MX4000	GK110-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK110-C
	Lightcycler 480 (96-well)	GK110-C