GeneQuery™ Human Innate Lymphoid Cell Development Markers qPCR Array Kit
(GQH-ILC)
Catalog #GK118

Product Description
ScienCell's GeneQuery™ Human Innate Lymphoid Cell Development Markers qPCR Array Kit (GQH-ILC) is designed to facilitate gene expression profiling of 24 marker genes involved in the differentiation of common lymphoid progenitors (CLPs) into innate lymphoid cells (ILCs), natural killer (NK) cells, and lymphoid tissue inducer (LTi) cells. Brief examples of how included genes may be grouped according to their functions are shown below:

- **CLP markers:** MME, CD34, IL7R, FLT3
- **Immature NK cell markers:** CD33, PROM1, CD34, KIT, IL2RB, KLRK1
- **Mature NK cell markers:** FCGR3A, IL2RA, NCAM1, B3GAT1, KLRD1, KLRC1, NCR1/2/3
- **ILC progenitor markers:** CD34, KIT, IL7R
- **ILC markers:** NCAM1, KLRD1, ITGAE, IL7R, KLRB1, PTPRC, NCR1, RORC
- **LTi cell markers:** CD4, IL7R, CCR6

Note: all gene names follow their official symbols by the Human Genome Organization Gene Nomenclature Committee (HGNC).

GeneQuery™ qPCR array kits are qPCR ready in a 96-well plate format, with each well containing one primer set that can specifically recognize and efficiently amplify a 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 target gene, unless otherwise indicated; 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 (ACTB, GAPDH, LDHA, NONO, and PPIH), which enable normalization of data.
- The Genomic DNA (gDNA) Control (GDC) detects possible gDNA contamination in the cDNA samples. It contains a primer set targeting a non-transcribed region of the genome.
- Positive PCR Control (PPC) 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) is strongly recommended, and can be used to monitor the DNA contamination introduced during the workflow such 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$_2$O	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 the primer sets are validated by qPCR with melt curve analysis. The PCR products are analyzed by gel electrophoresis. Single band amplification is confirmed for each set of primers.

Product Use
GQH-ILC 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
The 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 the plate 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$_2$O	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$_2$O 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	
Annealing	65°C	20 sec	40
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.

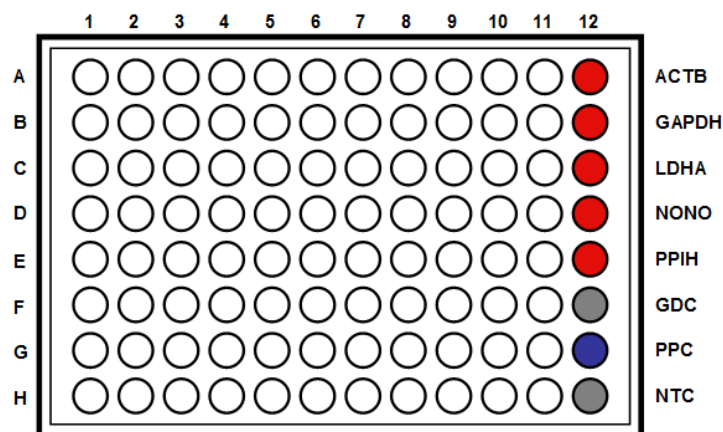Figure 1. Layout of GeneQuery™ qPCR array kit controls.

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)	$Cq \geq 35$	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.)

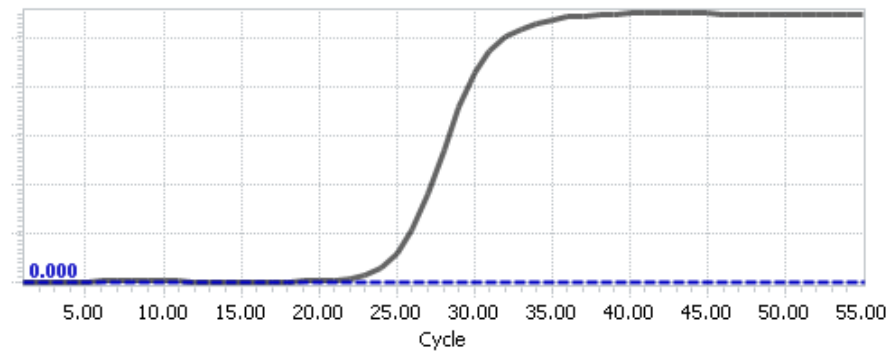Figure 2. A typical amplification curve showing the amplification of a qPCR product.
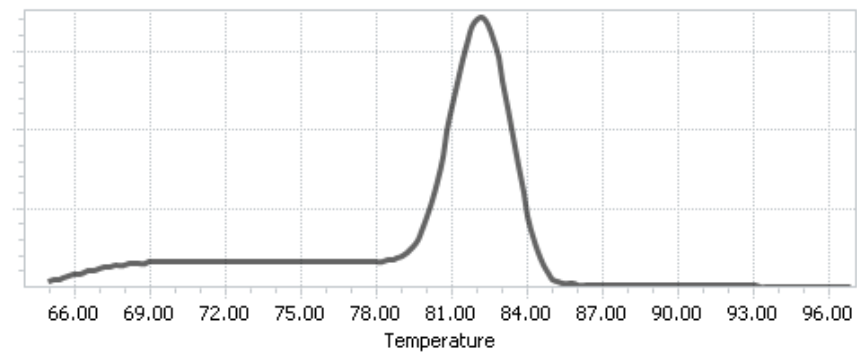

Figure 3. A typical melting peak of a qPCR product.

Quantification Method: Comparative ΔΔCq (Quantification Cycle Value) Method

1. **<u>Note</u>:** 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, ACTB, 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.

    ΔCq (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.

    ΔCq (ref) = average (ΔCq (HKG1), ΔCq (HKG2),......., ΔCq (HKG n)) (n is the number of housekeeping genes selected)

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

    ΔCq (ref) = (ΔCq(ACTB)+ΔCq(GAPDH)+ΔCq(LDHA)+ΔCq(NONO)+ΔCq(PPIH)) /5

    <u>Note</u>: 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),

    ΔCq (GOI) = Cq (GOI, experimental sample) - Cq (GOI, control sample)

    ΔΔCq = ΔCq (GOI) - ΔCq (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.

Samples	Genes of Interest		Housekeeping Genes				
	GOI1	GOI2	ACTB	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

ΔCq (ref) = (ΔCq(ACTB)+ΔCq(GAPDH)+ΔCq(LDHA)+ΔCq(NONO)+ΔCq(PPIH)) /5

$\qquad$ = ((17.16-18.20)+(17.84-18.48)+(20.12-20.57)+(19.64-19.50)+(26.40-26.55))/5

$\qquad$ = -0.43

ΔCq (GOI1) = 21.61 - 33.13

$\qquad$ = -11.52

ΔCq (GOI2) = 22.19 - 26.47

$\qquad$ = -4.28

$\Delta\Delta$Cq (GOI1) = ΔCq (GOI1) - ΔCq (ref)

$\qquad$ = -11.52 - (-0.43)

$\qquad$ = -11.09

$\Delta\Delta$Cq (GOI2) = ΔCq (GOI2) - ΔCq (ref)

$\qquad$ = -4.28 - (-0.43)

$\qquad$ = -3.85

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

$\qquad\qquad$ = $2^{11.09}$

$\qquad\qquad$ = 2180

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

$\qquad\qquad$ = $2^{3.85}$

$\qquad\qquad$ = 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™ Human Innate Lymphoid Cell Development Markers qPCR Array Plate Layout*
(**8 controls** in Bold and Italic)

Note: all gene names follow their official symbols by HGNC

	1	2	3	4	5	6	7	8	9	10	11	12
A	B3GAT1	IL2RB	MME	*ACTB*	B3GAT1	IL2RB	MME	*ACTB*	B3GAT1	IL2RB	MME	*ACTB*
B	CCR6	IL7R	NCAM1	*GAPDH*	CCR6	IL7R	NCAM1	*GAPDH*	CCR6	IL7R	NCAM1	*GAPDH*
C	CD33	ITGAE	NCR1	*LDHA*	CD33	ITGAE	NCR1	*LDHA*	CD33	ITGAE	NCR1	*LDHA*
D	CD34	KIT	NCR2	*NONO*	CD34	KIT	NCR2	*NONO*	CD34	KIT	NCR2	*NONO*
E	CD4	KLRB1	NCR3	*PPIH*	CD4	KLRB1	NCR3	*PPIH*	CD4	KLRB1	NCR3	*PPIH*
F	FCGR3A	KLRC1	PROM1	*GDC*	FCGR3A	KLRC1	PROM1	*GDC*	FCGR3A	KLRC1	PROM1	*GDC*
G	FLT3	KLRD1	PTPRC	*PPC*	FLT3	KLRD1	PTPRC	*PPC*	FLT3	KLRD1	PTPRC	*PPC*
H	IL2RA	KLRK1	RORC	*NTC*	IL2RA	KLRK1	RORC	*NTC*	IL2RA	KLRK1	RORC	*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	GK118-A
	ABI 7000	GK118-A
	ABI 7300	GK118-A
	ABI 7500	GK118-A
	ABI 7700	GK118-A
	ABI 7900 HT	GK118-A
	QuantStudio	GK118-A
	ViiA 7	GK118-A
Bio-Rad	Chromo4	GK118-A
	iCycler	GK118-A
	iQ5	GK118-A
	MyiQ	GK118-A
	MyiQ2	GK118-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK118-A
	Matercycler ep realplex 4	GK118-A
Stratagene	MX3000P	GK118-A
	MX3005P	GK118-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK118-B
	ABI 7900 HT Fast	GK118-B
	QuantStudio Fast	GK118-B
	StepOnePlus	GK118-B
	ViiA 7 Fast	GK118-B
Bio-Rad	CFX Connect	GK118-B
	CFX96	GK118-B
	DNA Engine Opticon 2	GK118-B
Stratagene	MX4000	GK118-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK118-C
	Lightcycler 480 (96-well)	GK118-C