GeneQuery™ Human Regulation of Cancer Immune Evasion qPCR Array Kit (Plate 1 of 2) (GQH-CIE1)

Catalog #GK134

Product Description

The immune system can effectively recognize cancer cells. However, a hallmark of cancer cells is they acquire the ability to evade immune destruction. The genetic basis for cancer immune evasion remains largely elusive. Cancer immunotherapy, an emerging and effective way to treat many types of cancer, utilizes the ability of the immune system to recognize cancer cells while depriving cancer cells of their immune evasion. ScienCell's GeneQuery™ Human Regulation of Cancer Immune Evasion qPCR Array kits (GQH-CIE1 and GQH-CIE2) are designed to facilitate a better understanding of the mechanisms by which cancer cells evade immune control. The genes selected have been shown to enhance or decrease the resistance of cancer cells to immune control when disrupted. Genes selected are based on genome-wide CRISPR screens[1], and brief examples of how included genes may be grouped according to their functions are shown below:

- **Necroptosis:** FADD, FAS, TNFRSF1A, TRADD, TRAF2, FAS, IRF9, CFLAR, TRPM7, IFNAR1, IFNAR2, IFNGR1, IFNGR2, RBCK1, TNFAIP3, RNF31, VPS4B, CHMP5, VDAC2
- **Autophagy:** MAPK1, MAP3K7, CFLAR, BECN1, ATG12, ATG7, ATG5, ATG3, RB1CC1, ATG14, ATG9A, ATG101, BECN1, ATG10, ATG14
- **Endocytosis:** TGFBR2, TFRC, ARF6, VPS4B, CHMP5, VPS35, IST1
- **TNF signaling:** MAP3K7, FADD, TNFRSF1B, TNFRSF1A, TNFAIP3, TRADD, CFLAR, MAPK1, TAB2, FAS, TRAF2, TAB1, BCL2L1, CREBBP, TBK1, MAPK1, FAS, IRF1
- **Interferon signaling:** IFNAR1, IFNGR1, IFNGR2, STAT1, JAK1, JAK2, PTPN2, STAT2, IFNAR2, SOCS1, UBE2N, IRF9, IRF1, TAP1, PSMB9
- **NOD-like receptor signaling:** FADD, TRAF2, MAPK1, IRF9, TRPM7, IFNAR1, IFNAR2, ATG12, ATG5, RBCK1, JAK1, TNFAIP3, RNF31, VDAC2, PKN2
- **NFKB signaling:** IKBKB, IKBKG, TNFRSF1A, TRADD, TRAF2, TAB1, TAB2, MAP3K7, CFLAR, TNFAIP3
- **JAK-STAT signaling:** JAK2, IRF9, SOCS1, IFNAR1, IFNAR2, IFNGR1, IFNGR2, JAK1, STAT1, STAT2, PTPN2, PKN2
- **MAPK signaling:** TNFRSF1A, TRADD, TRAF2, MAPK1, TGFBR2, FAS, TAB1, TAB2, MAP3K7, MAPK1, FAS, TAB2, TAB1, PKN2
- **IL-17 signaling:** FADD, TRADD, TRAF2, MAPK1, TNFAIP3
- **Th17 cell differentiation:** MAPK1, TGFBR2, JAK2, IFNGR1, IFNGR2, JAK1, STAT1
- **GPI anchor biosynthesis:** PIGK, PIGS, PIGT, PIGU, GPAA1
- **Antigen presentation:** TAP2, B2M, TAP1, CALR, PDIA3, TAPBP
- **Ubiquitin mediated proteolysis:** CUL3, UBR5, SOCS1, UBE2N, UBE2G2, RBCK1, ATG7, UFL1

- **Protein processing in ER:** UBE2G2, MOGS, PRKCSH, NPLOC4, TRAF2, PDIA3, CALR
- **Other innate immunity signaling pathways:** HDAC1, SRSF7, PPP1CA, HCFC2, USP7, ADAR, NXT1, PPP2R2A, PPP2R3C

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	Cat #	Quantity	Storage
GeneQuery™ array plate with lyophilized primers	GK134	1	4°C or -20°C
Optical PCR plate seal	N/A	1	RT
Nuclease-free H_2O	GQ100-1	2	4°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
Reverse transcription kit	First-Strand cDNA Synthesis Master Mix, 4x (ScienCell, Cat #MB6008)
cDNA template	Customers' samples
qPCR master mix	GoldNStart TaqGreen qPCR Master Mix (ScienCell, Cat #MB6018)

References

[1] K. A. Lawson *et al.* Functional genomic landscape of cancer-intrinsic evasion of killing by T cells. ***Nature,*** 586, 120-6 (2020)

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-CIE1 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_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.

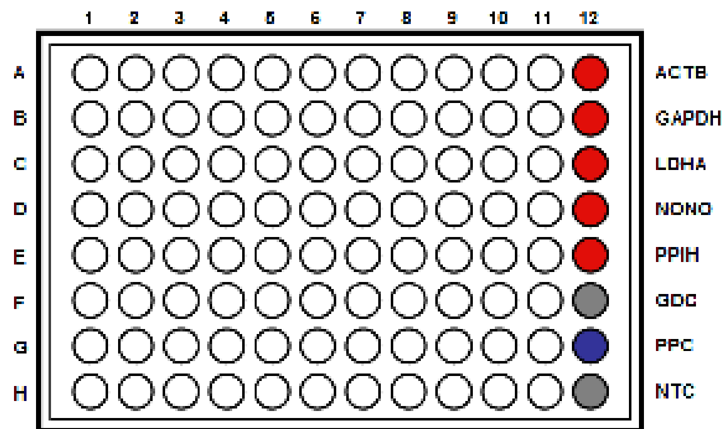


Table 2. Interpretation of control results:

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.

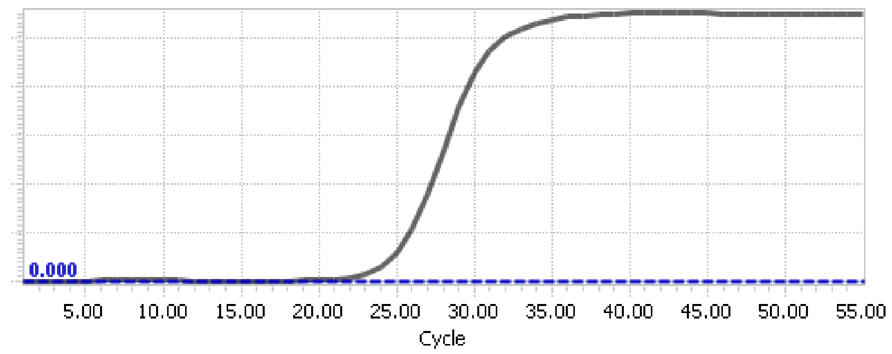
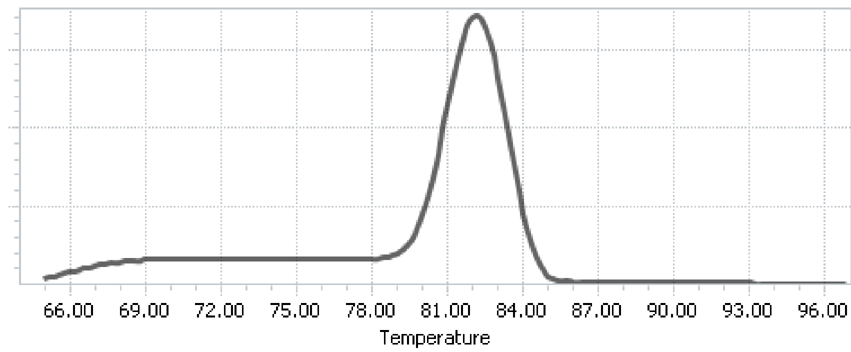


Figure 3. A typical melting peak of a qPCR product.



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: 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

   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),

   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.

	Genes of Interest		Housekeeping Genes				
Samples	**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

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

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

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

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

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

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.

Rev. 1

GeneQuery™ Human Regulation of Cancer Immune Evasion qPCR Array Kit (Plate 1 of 2) (GQH-CIE1)

Catalog #GK134

GeneQuery™ Human Regulation of Cancer Immune Evasion qPCR Array Kit (Plate 1 of 2) Plate Layout*
(***8 controls*** in Bold and Italic)

	1	2	3	4	5	6	7	8	9	10	11	12
A	ACAD9	ATG101	ATXN7L3	CAD	CUL3	EIF3H	F8A1	GPAA1	IFNAR1	IRF1	KLF16	***ACTB***
B	ACTB	ATG12	B2M	CALR	DCP1A	EMC2	FADD	PIGQ	IFNAR2	IRF9	KMT2A	***GAPDH***
C	ADAR	ATG14	ODR4	CEP55	DET1	EMC3	CCNQ	HCFC2	IFNGR1	IST1	LIPT2	***LDHA***
D	AGO2	ATG3	BCL2L1	CFLAR	DICER1	EMC4	FAS	HDAC1	IFNGR2	JAGN1	MAP3K7	***NONO***
E	AHSA1	ATG5	BECN1	CHIC2	DNAJC13	EMC6	FITM2	HDGFL2	IKBKB	JAK1	MAPK1	***PPIH***
F	ANAPC15	ATG7	BOLA3	CHMP5	DNTTIP1	EMC8	FNTB	HEXIM1	IKBKG	JAK2	MED16	***GDC***
G	ARF6	ATG9A	BRAT1	COX6C	DOT1L	ERAP1	GALE	HIRA	INO80	JMJD6	MED23	***PPC***
H	ATG10	ATP13A1	BRPF1	CREBBP	DPH5	ERP44	GLS	HSPA13	IPPK	KAT6A	MED24	***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	GK134-A
	ABI 7000	GK134-A
	ABI 7300	GK134-A
	ABI 7500	GK134-A
	ABI 7700	GK134-A
	ABI 7900 HT	GK134-A
	QuantStudio	GK134-A
	ViiA 7	GK134-A
Bio-Rad	Chromo4	GK134-A
	iCycler	GK134-A
	iQ5	GK134-A
	MyiQ	GK134-A
	MyiQ2	GK134-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK134-A
	Matercycler ep realplex 4	GK134-A
Stratagene	MX3000P	GK134-A
	MX3005P	GK134-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK134-B
	ABI 7900 HT Fast	GK134-B
	QuantStudio Fast	GK134-B
	StepOnePlus	GK134-B
	ViiA 7 Fast	GK134-B
Bio-Rad	CFX Connect	GK134-B
	CFX96	GK134-B
	DNA Engine Opticon 2	GK134-B
Stratagene	MX4000	GK134-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK134-C
	Lightcycler 480 (96-well)	GK134-C