Rev.1

GeneQuery™ Human Non-Small Cell Lung Cancer qPCR Array Kit (GQH-NLC)

Catalog #GK033

Product Description

ScienCell's GeneQuery™ Human Non-Small Cell Lung Cancer qPCR Array Kit (GQH-NLC) is designed to facilitate gene expression profiling of 88 key genes involved in non-small cell lung cancer (NSCLC) carcinogenesis. NSCLC accounts for more than 80% of all lung cancers. There are three main types of NSCLC: adenocarcinoma, lung squamous cell carcinoma and lung large cell carcinoma. Brief examples of how included genes may be grouped according to their functions are shown below:

- **Oncogenes and tumor suppressor genes:** KRAS, HRAS, EML4, ALK, RARB, FHIT, RASSF1, TP53, CDKN2A
- **Cell cycle regulation and apoptosis:** CDK4, CDK6, CCND1, CCNE2, CASP3, CASP9, BAD, STK4, FOXO3
- **MAPK pathway:** KRAS, HRAS, NRAS, RAF1, MAP2K1, MAPK1, MAPK3
- **PI3K/AKT pathway:** PI3Ks, AKT1, AKT2
- **Genes related to specific lung cancer types**
    - **Adenocarcinoma:** ACTR3B, CADM1, ERBB2, CALB2, CDH1, EGF, FIGF, KRT20, NKX2-1, OGG1
    - **Lung squamous cell carcinoma:** CDH10, CYP1A1, FAM83B, FGFR1, GRAMD4, KRT19, PEBP1, PRKCI, S100A2, SOX2
    - **Lung large cell carcinoma:** CASK, CDC42BPB, DDR2, EPHA3, MYO18B, NME1, ROS1, TTN, WNK3

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	GK033	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 transcriptase	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)

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-NLC 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.

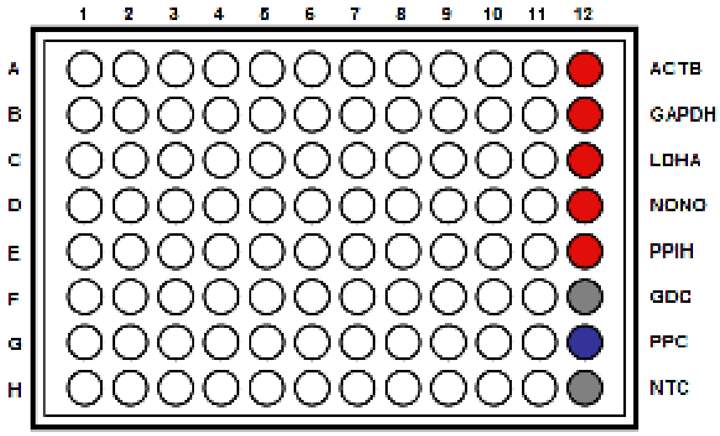


Table 2. Interpretation of control results:

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.

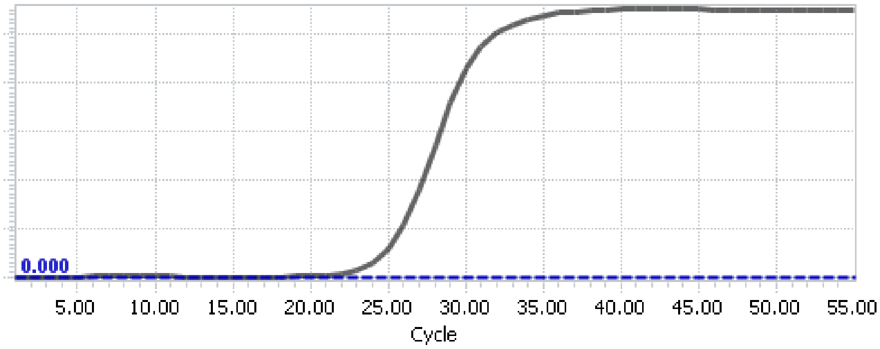
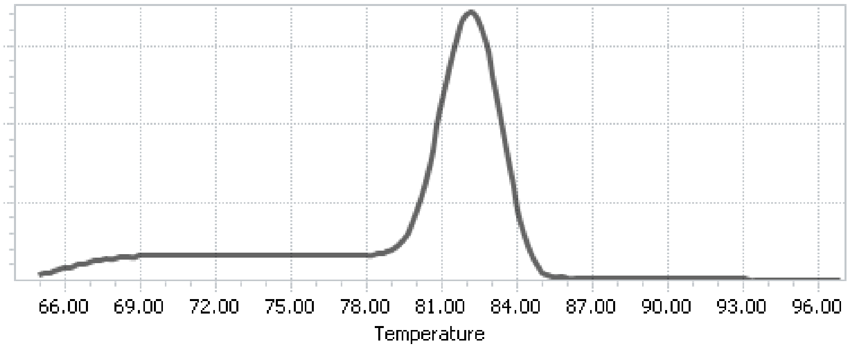


Figure 3. A typical melting peak of a qPCR product.



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.

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

   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.

   $\Delta Cq\ (ref) = average\ (\Delta Cq\ (HKG1), \Delta Cq\ (HKG2), \ldots\ldots., \Delta Cq\ (HKG\ n))$ (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:

   $$\Delta Cq\ (ref) = (\Delta Cq(ACTB)+\Delta Cq(GAPDH)+\Delta Cq(LDHA)+\Delta Cq(NONO)+\Delta Cq(PPIH))\ /5$$

   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),

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

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

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

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.

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GeneQuery™ Human Non-Small Cell Lung Cancer qPCR Array Plate Layout*
(***8 controls*** in Bold and Italic)

	1	2	3	4	5	6	7	8	9	10		
A	ACTR3B	CALB2	CDK4	EPHA3	GRAMD4	MAP2K2	NRAS	PIK3R1	RAF1	RXRA	SOX2	***ACTB***
B	AKT1	CASK	CDK6	EPHA5	GRB2	MAPK1	NTRK3	PIK3R3	RAP2B	RXRG	STK11	***GAPDH***
C	AKT2	CASP3	CDKN2A	ERBB2	HRAS	MAPK3	OGG1	PIK3R5	RARB	S100A2	STK4	***LDHA***
D	ALK	CASP9	CYP1A1	FAM83B	KRAS	MMP2	PDPK1	PLCG2	RASSF1	S100A7	TGFA	***NONO***
E	ARAF	CCND1	DDR2	FGFR1	KRT19	MUC1	PEBP1	PRKCG	RASSF5	SFTPB	TP53	***PPIH***
F	BAD	CDC42BPB	E2F2	FHIT	KRT20	MYO18B	PIK3CA	PRKCI	RB1	SFTPC	TTN	***GDC***
G	BRAF	CDH1	EGF	FIGF	KRT7	NKX2-1	PIK3CD	PTGS2	RBMS3	SOS1	VEGFA	***PPC***
H	CADM1	CDH10	EML4	FOXO3	MAP2K1	NME1	PIK3CG	PTHLH	ROS1	SOS2	WNK3	***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	GK033-A
	ABI 7000	GK033-A
	ABI 7300	GK033-A
	ABI 7500	GK033-A
	ABI 7700	GK033-A
	ABI 7900 HT	GK033-A
	QuantStudio	GK033-A
	ViiA 7	GK033-A
Bio-Rad	Chromo4	GK033-A
	iCycler	GK033-A
	iQ5	GK033-A
	MyiQ	GK033-A
	MyiQ2	GK033-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK033-A
	Matercycler ep realplex 4	GK033-A
Stratagene	MX3000P	GK033-A
	MX3005P	GK033-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK033-B
	ABI 7900 HT Fast	GK033-B
	QuantStudio Fast	GK033-B
	StepOnePlus	GK033-B
	ViiA 7 Fast	GK033-B
Bio-Rad	CFX Connect	GK033-B
	CFX96	GK033-B
	DNA Engine Opticon 2	GK033-B
Stratagene	MX4000	GK033-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK033-C
	Lightcycler 480 (96-well)	GK033-C