GeneQuery™ Human Melanoma qPCR Array Kit
(GQH-MEL)
Catalog #GK012

Product Description

ScienCell's GeneQuery™ Human Melanoma qPCR array kit (GQH-MEL) is designed to facilitate gene expression profiling of human melanoma related genes. Melanoma, also known as malignant melanoma, is a type of malignant cancer that results from uncontrolled growth of melanocytes, which are melanin-producing, pigmented cells. 88 genes are selected in this kit based on database and literature research.

GeneQuery™ PCR 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_2O	2 mL	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-MEL 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	
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.

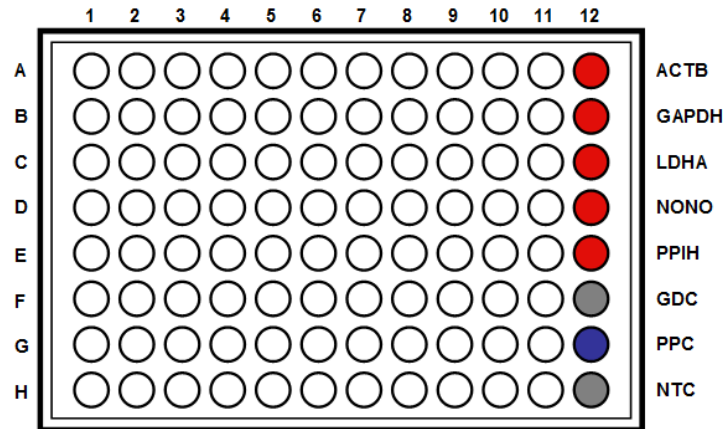Figure 1. Layout of GeneQuery™ qPCR array kit controls.

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.)

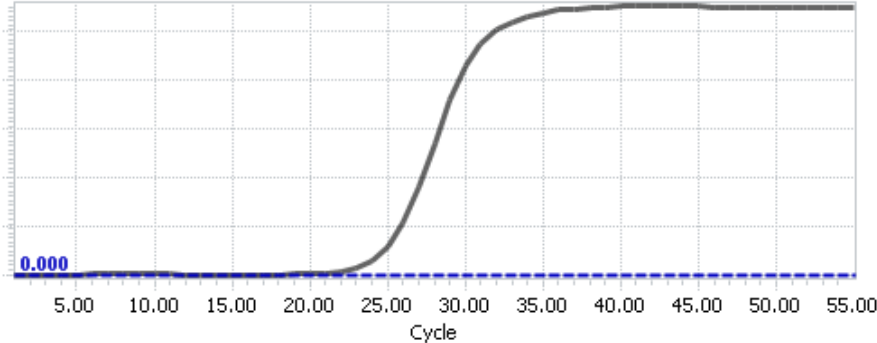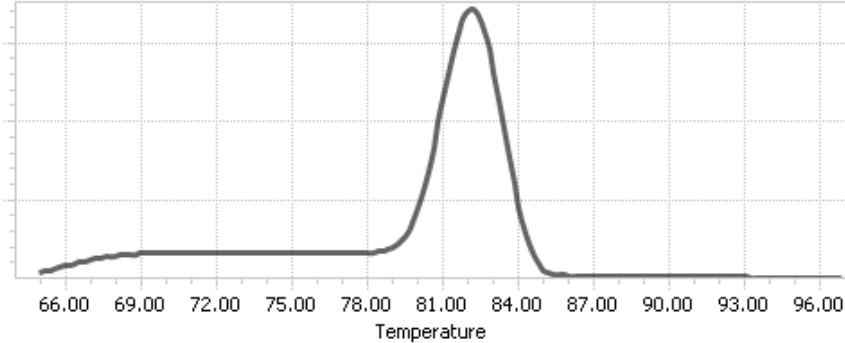Figure 2. A typical amplification curve showing the amplification of a qPCR product.

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

Quantification Method: Comparative ΔΔCq (Quantification Cycle Value) Method

1. **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

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 = $2^{-\Delta\Delta Cq \, (GOI1)}$

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

$\qquad\qquad\qquad\qquad\qquad\qquad\qquad = 2180$

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

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

$\qquad\qquad\qquad\qquad\qquad\qquad\qquad = 14.4$

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

GeneQuery™ Human Melanoma qPCR Array Plate Layout* (*8 controls* in Bold and Italic)

	1	2	3	4	5	6	7	8	9	10	11	12
A	ABCC2	BRAF	CTGF	EGFR	GNAQ	ITGA7	MITF	OPN	PPP2R2C	SHOX2	TM4SF13	*ACTB*
B	AKT1	BRCA2	CTNNB1	FABP7	HOXB7	ITGB3	MLANA	MAPK14	PTPRF	SNAI2	TMEM229B	*GAPDH*
C	AKT2	BRN2	CXCR4	FGF13	HRAS	KIT	MX1	PAX3	Rab5b	SNAI1	TNC	*LDHA*
D	AKT3	CDH1	CYR61	FGFR2	IGF1	KRAS	NDN	PIK3CA	RB1	SOS1	TP53	*NONO*
E	ASXL1	CDKN2A	DNAJA1	FGFR3	IGFBP3	LIF	NOTCH2	PIK3CB	RNF128	SOX10	TPD52	*PPIH*
F	BCL2	CHP2	DTX3L	FOXD1	ISG15	MAPK3	NPM2	PIK3CD	RPS6KA1	STK11	TYRP1	*GDC*
G	BCL2L1	COL11A1	EDNRB	FOXD3	ITGA4	MC1R	NRAS	PLA1A	RUNX3	TIMP3	UCHL1	*PPC*
H	BMI	CSPG4	ERCC2	GAPDHS	ITGA6	MCAM	OAS1	PMEL	SHC4	TM4SF1	WNT4	*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	GK012-A
	ABI 7000	GK012-A
	ABI 7300	GK012-A
	ABI 7500	GK012-A
	ABI 7700	GK012-A
	ABI 7900 HT	GK012-A
	QuantStudio	GK012-A
	ViiA 7	GK012-A
Bio-Rad	Chromo4	GK012-A
	iCycler	GK012-A
	iQ5	GK012-A
	MyiQ	GK012-A
	MyiQ2	GK012-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK012-A
	Matercycler ep realplex 4	GK012-A
Stratagene	MX3000P	GK012-A
	MX3005P	GK012-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK012-B
	ABI 7900 HT Fast	GK012-B
	QuantStudio Fast	GK012-B
	StepOnePlus	GK012-B
	ViiA 7 Fast	GK012-B
Bio-Rad	CFX Connect	GK012-B
	CFX96	GK012-B
	DNA Engine Opticon 2	GK012-B
Stratagene	MX4000	GK012-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK012-C
	Lightcycler 480 (96-well)	GK012-C