



## 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 public 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<sup>2+</sup>, 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 ( $\beta$ -actin, 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 <sub>2</sub> 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)
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**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

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**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  $\mu$ l PCR reactions for one well as shown in Table 1.

Table 1

<b>cDNA template</b>	<b>0.2 – 250 ng</b>
2x qPCR master mix	10 $\mu$ l
Nuclease-free H <sub>2</sub> O	variable
<b>Total volume</b>	<b>20 <math>\mu</math>l</b>

**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<sub>2</sub>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 H<sub>2</sub>O 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		
<i>Recommended</i>	<i>Melting curve analysis</i>		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.

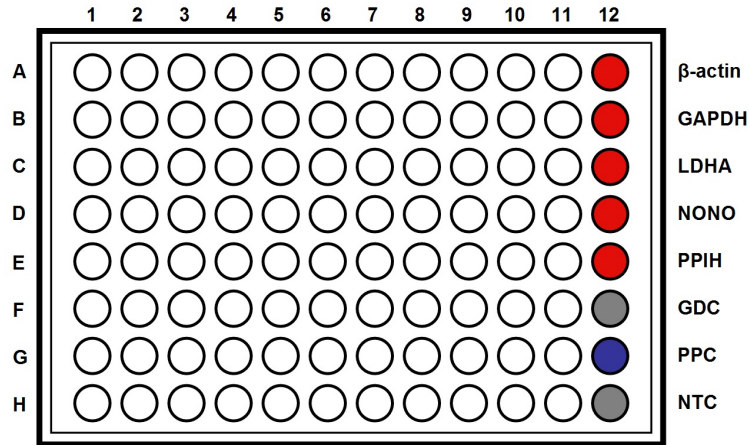


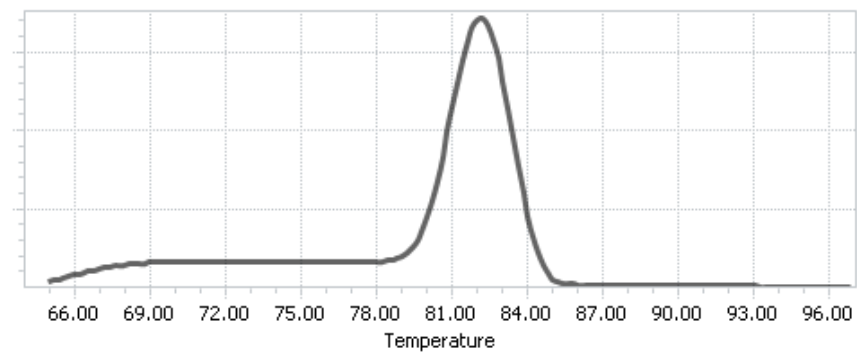
Table 2. Interpretation of control results:

<i>Controls</i>	<i>Results</i>	<i>Interpretation</i>	<i>Suggestions</i>
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.





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

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>
<b>A</b>	ABCC2	BRAF	CTGF	EGFR	GNAQ	ITGA7	MITF	OPN	PPP2R2C	SHOX2	TM4SF13	<i><b><math>\beta</math>-actin</b></i>
<b>B</b>	AKT1	BRCA2	CTNNB1	FABP7	HOXB7	ITGB3	MLANA	MAPK14	PTPRF	SNAI2	TMEM229B	<i><b>GAPDH</b></i>
<b>C</b>	AKT2	BRN2	CXCR4	FGF13	HRAS	KIT	MX1	PAX3	Rab5b	SNAI1	TNC	<i><b>LDHA</b></i>
<b>D</b>	AKT3	CDH1	CYR61	FGFR2	IGF1	KRAS	Necdin	PIK3CA	RB1	SOS1	TP53	<i><b>NONO</b></i>
<b>E</b>	ASXL1	CDKN2A	DNAJA1	FGFR3	IGFBP3	LIF	NOTCH2	PIK3CB	RNF128	SOX10	TPD52	<i><b>PPIH</b></i>
<b>F</b>	BCL2	CHP2	DTX3L	FOXD1	ISG15	MAPK3	NPM2	PIK3CD	RPS6KA1	STK11	TYRP1	<i><b>GDC</b></i>
<b>G</b>	BCL2L1	COL11A1	EDNRB	FOXD3	ITGA4	MC1R	NRAS	PLA1A	RUNX3	TIMP3	UCHL1	<i><b>PPC</b></i>
<b>H</b>	BMI	CSPG4	ERCC2	GAPDHS	ITGA6	MCAM	OAS1	PMEL	SHC4	TM4SF1	WNT4	<i><b>NTC</b></i>

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