



**GeneQuery™ Human Endothelial Cell Heterogeneity and Differentiation  
qPCR Array Kit  
(GQH-EHD)  
Catalog #GK023**

**Product Description**

ScienCell's GeneQuery™ Human Endothelial Cell Heterogeneity and Differentiation qPCR Array Kit (GQH-EHD) is designed to facilitate gene expression profiling of 88 key genes to help characterize endothelial cells during lineage differentiation and is based on extensive literature research. Brief examples of how included genes may be grouped according to specification markers are shown below:

- **Circulating Early Endothelial Progenitors:** MAFB, RB1, ADORA3, MYCL1, NCOA2
- **Circulating Late Endothelial Progenitors:** FOXF1, ITGAM, SIX4, EGFR, CREB5
- **Hemangioblasts:** GATA1, LMO2, PROM1, RUNX1, TAL1
- **Lymphatic Endothelial Cells:** FLT4, LYVE1, PDPN, PROX1, FGFR3
- **EndMT:** ACTA2, SMAD3, S100A4, TWIST, BMP7

GeneQuery™ qPCR array kits are qPCR ready in a 96-well plate format, with each well containing one primer set that recognizes and efficiently amplifies a specific 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 the target gene, unless otherwise noted; 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), which detects gDNA contamination in cDNA samples. This primer set targets a non-transcribed region of the genome.
- Positive PCR Control (PPC), which tests whether samples contain inhibitors or other factors that may negatively affect gene expression results. The PPC consists of a predisposed 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), which can be used to monitor DNA contamination introduced during workflow (e.g. from such sources 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)

### Quality Control

All primer sets are validated by qPCR with melt curve analysis and analyzed by gel electrophoresis. Single band amplification is confirmed for each set of primers.

### Product Use

GQH-ANG 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

This 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 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><i>Total volume</i></b>	<b><i>20 <math>\mu</math>l</i></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

<b>Step</b>	<b>Temperature</b>	<b>Time</b>	<b>Number of cycles</b>
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.

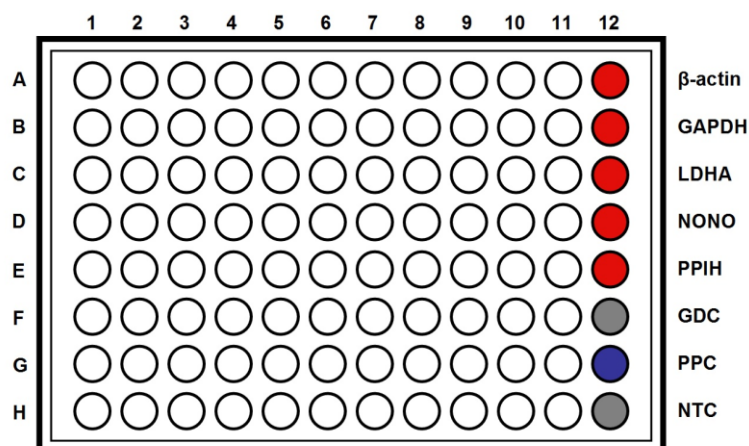


Table 2. Interpretation of control results:

<i>Controls</i>	<i>Results</i>	<i>Interpretation</i>	<i>Suggestions</i>
Housekeeping gene controls	Variability of a housekeeping gene's C <sub>q</sub> 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)	C <sub>q</sub> ≥ 35	No gDNA detected	N/A
	C <sub>q</sub> < 35	The sample is contaminated with gDNA	Perform DNase digestion during RNA purification step
Positive PCR Control (PPC)	C <sub>q</sub> > 30; or The C <sub>q</sub> 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.

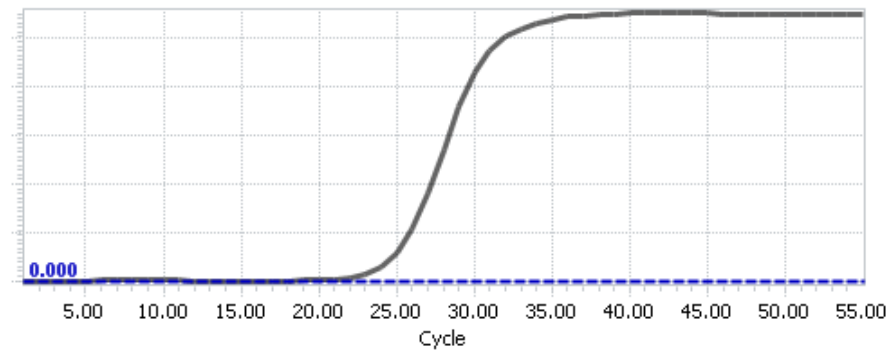
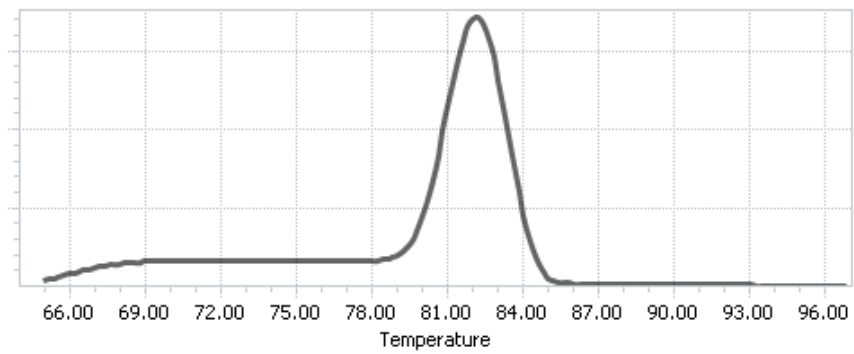


Figure 3. A typical melting peak of a qPCR product.



## **Quantification Method: Comparative $\Delta\Delta Cq$ (Quantification Cycle Value) Method**

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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,  $\beta$ -actin, GAPDH, LDHA, NONO, and PPIH.

2. For a single housekeeping gene,  $\Delta Cq$  (ref) is the quantification cycle number change for that housekeeping gene (HKG) between an experimental sample and control sample.

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

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  $\Delta Cq$  of the selected housekeeping genes.

$\Delta Cq \text{ (ref)} = \text{average} (\Delta Cq \text{ (HKG1)}, \Delta Cq \text{ (HKG2)}, \dots, \Delta Cq \text{ (HKG n)})$  (n is the number of housekeeping genes selected)

**If** using all 5 housekeeping genes included in this kit,  $\beta$ -actin, GAPDH, LDHA, NONO, and PPIH, use the following formula:

$$\Delta Cq \text{ (ref)} = (\Delta Cq(\beta\text{-actin}) + \Delta Cq(\text{GAPDH}) + \Delta Cq(\text{LDHA}) + \Delta Cq(\text{NONO}) + \Delta Cq(\text{PPIH})) / 5$$

**Note:**  $\Delta Cq \text{ (HKG)} = Cq \text{ (HKG, experimental sample)} - Cq \text{ (HKG, control sample)}$ , and  $\Delta Cq \text{ (HKG)}$  value can be positive, 0, or negative.

3. For any of your genes of interest (GOI),

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

$$\Delta\Delta Cq = \Delta Cq \text{ (GOI)} - \Delta Cq \text{ (ref)}$$

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

## **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 $\Delta\Delta Cq$ (Quantification Cycle Value) Method

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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	<i><math>\beta</math>-actin</i>	<i>GAPDH</i>	<i>LDHA</i>	<i>NONO</i>	<i>PPIH</i>
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

$$\begin{aligned}\Delta Cq(\text{ref}) &= (\Delta Cq(\beta\text{-actin}) + \Delta Cq(\text{GAPDH}) + \Delta Cq(\text{LDHA}) + \Delta Cq(\text{NONO}) + \Delta Cq(\text{PPIH})) / 5 \\ &= ((17.16 - 18.20) + (17.84 - 18.48) + (20.12 - 20.57) + (19.64 - 19.50) + (26.40 - 26.55)) / 5 \\ &= -0.43\end{aligned}$$

$$\begin{aligned}\Delta Cq(\text{GOI1}) &= 21.61 - 33.13 \\ &= -11.52\end{aligned}$$

$$\begin{aligned}\Delta Cq(\text{GOI2}) &= 22.19 - 26.47 \\ &= -4.28\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq(\text{GOI1}) &= \Delta Cq(\text{GOI1}) - \Delta Cq(\text{ref}) \\ &= -11.52 - (-0.43) \\ &= -11.09\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq(\text{GOI2}) &= \Delta Cq(\text{GOI2}) - \Delta Cq(\text{ref}) \\ &= -4.28 - (-0.43) \\ &= -3.85\end{aligned}$$

$$\begin{aligned}\text{Normalized GOI1 expression level fold change} &= 2^{-\Delta\Delta Cq(\text{GOI1})} \\ &= 2^{11.09} \\ &= 2180\end{aligned}$$

$$\begin{aligned}\text{Normalized GOI2 expression level fold change} &= 2^{-\Delta\Delta Cq(\text{GOI2})} \\ &= 2^{3.85} \\ &= 14.4\end{aligned}$$

**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 Endothelial Cell Heterogeneity and Differentiation 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>	ACE	CD34	EGFR	FOXF1	IL1R1	LYVE1	NFYA	PODXL	RUNX1	SLC2A9	TAL1	<i><b>β-actin</b></i>
<b>B</b>	ACTA2	CD36	EPAS1	GATA1	ITGAM	MAFB	NODAL	PROM1	S100A4	SMAD1	TBX18	<i><b>GAPDH</b></i>
<b>C</b>	ADORA3	CDH1	EFNB2	GATA6	JAG1	MAP3K10	NOTCH1	PROX1	SEMA6D	SMAD3	TEK	<i><b>LDHA</b></i>
<b>D</b>	BMP10	CDH5	EPHB4	GDF2	JDP2	MAPK14	NR2F2	PTGFR	SHH	SMAD5	TGFBR3	<i><b>NONO</b></i>
<b>E</b>	BMP4	CFC1	ETV1	GSC	KDR	MCAM	NRP1	RB1	SIGLEC1	SNAI1	TRPS1	<i><b>PPIH</b></i>
<b>F</b>	BMP7	CREB5	FGFR3	GSK3B	LILRB5	MYCL	NRP2	RHOA	SIX4	SOX18	TWIST1	<i><b>GDC</b></i>
<b>G</b>	CD163	CTNNB1	FLT1	HDAC5	LMO2	NCOA2	PDPN	ROCK1	SLAMF8	T	VCAM1	<i><b>PPC</b></i>
<b>H</b>	CD248	CXADR	FLT4	ICAM2	LPAR1	NFATC1	PECAM1	ROR2	SLC16A3	TAGLN	ZEB1	<i><b>NTC</b></i>

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



## Appendix. Plate type choice chart.

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### Plate type A

Brand	Model	kit catalog #
ABI / Life Tech	ABI 5700	GK023-A
	ABI 7000	GK023-A
	ABI 7300	GK023-A
	ABI 7500	GK023-A
	ABI 7700	GK023-A
	ABI 7900 HT	GK023-A
	QuantStudio	GK023-A
	ViiA 7	GK023-A
Bio-Rad	Chromo4	GK023-A
	iCycler	GK023-A
	iQ5	GK023-A
	MyiQ	GK023-A
	MyiQ2	GK023-A
Eppendorf / Life Tech	Matercyler ep realplex 2	GK023-A
	Matercyler ep realplex 4	GK023-A
Stratagene	MX3000P	GK023-A
	MX3005P	GK023-A

### Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK023-B
	ABI 7900 HT Fast	GK023-B
	QuantStudio Fast	GK023-B
	StepOnePlus	GK023-B
	ViiA 7 Fast	GK023-B
Bio-Rad	CFX Connect	GK023-B
	CFX96	GK023-B
	DNA Engine Opticon 2	GK023-B
Stratagene	MX4000	GK023-B

### Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK023-C
	Lightcycler 480 (96-well)	GK023-C