



**GeneQuery™ Human VEGF Signaling Pathway qPCR Array Kit  
(GQH-VEG)  
Catalog #GK088**

**Product Description**

ScienCell's GeneQuery™ Human Signaling Pathway qPCR Array Kit (GQH-VEG) profiles 88 key genes involved in VEGF and VEGF-related signaling. VEGF is a growth factor known for stimulating angiogenesis and vasculogenesis. As such, it is integral to proper blood vasculature formation during development, maintenance, and wound healing. Aberrant VEGF signaling is associated with ischemia, diabetic retinopathy, macular degeneration, and solid tumor progression. Brief examples of how included genes may be grouped according to their function are shown below:

- **Signaling receptors:** FLT1, FLT4, KDR, KIT, FGFR1
- **Growth factor ligands:** VEGFA, VEGFB, VEGFC, FGF2, PGF
- **Cytoskeletal response:** ACTA1, ACTA2, ACTB, ACTG1, ACTG2
- **Adhesion regulation:** ITGAV, ITGB1, ITGB3, ITGB5, PTK2
- **Downstream transcription factors:** ATF2, FOS, JUN, KRAS, NRAS

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 (ACTB, 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**

<b>Component</b>	<b>Cat #</b>	<b>Quantity</b>	<b>Storage</b>
GeneQuery™ array plate with lyophilized primers	GK088	1	4°C or -20°C
Optical PCR plate seal	N/A	1	RT
Nuclease-free H <sub>2</sub> O	GQ100-1	2	4°C

**Additional Materials Required (Materials Not Included in Kit)**

<b>Component</b>	<b>Recommended</b>
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 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-VEG 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>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.

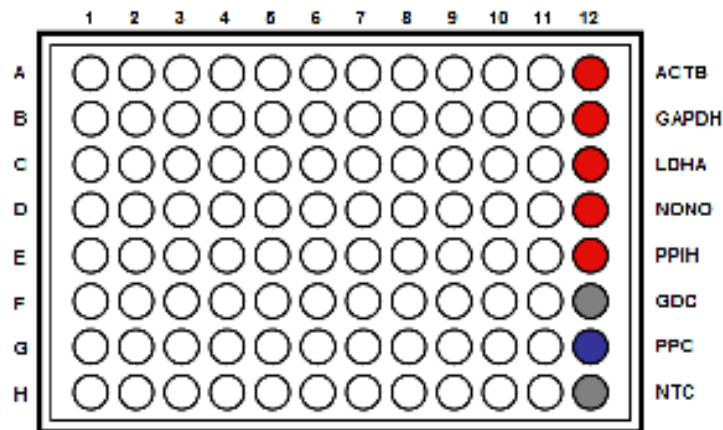


Table 2. Interpretation of control results:

<i>Controls</i>	<i>Results</i>	<i>Interpretation</i>	<i>Suggestions</i>
Housekeeping gene controls	Variability of a housekeeping gene's 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.)

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

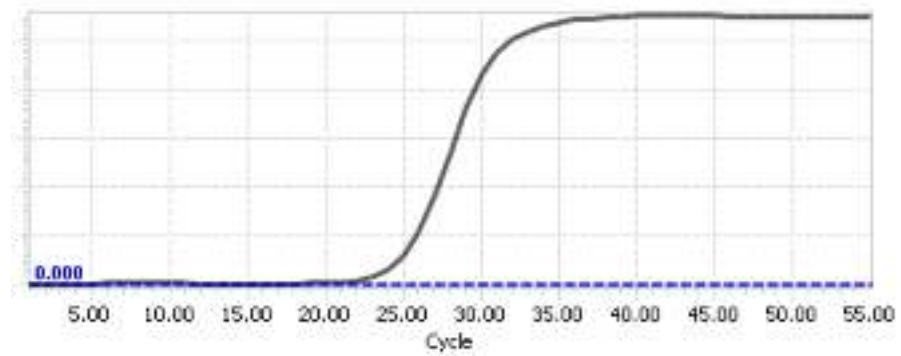
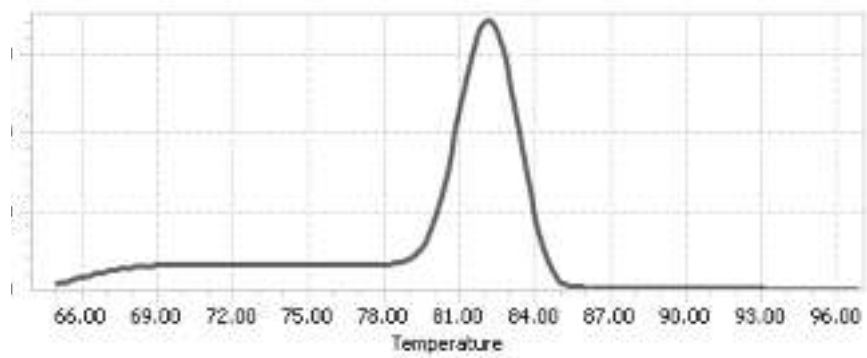


Figure 3. A typical melting peak of a qPCR product.



## **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: ACTB, 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 (ACTB, GAPDH, LDHA, NONO, and PPIH) use the following formula:

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

**Note:**  $\Delta Cq$  (HKG) =  $Cq$  (HKG, experimental sample) -  $Cq$  (HKG, control sample), and  $\Delta Cq$  (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**

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>ACTB</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

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

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

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

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

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

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

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

**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 VEGF Signaling Pathway 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>	ACTA1	ATF2	FGF2	FN1	ITGB3	L1CAM	MAPK3	NRP2	PRKCA	PRKCZ	SOS1	<b><i>ACTB</i></b>
<b>B</b>	ACTA2	BAD	FGFR1	FOS	ITGB5	MAP2K1	MAPK8	PGF	PRKCB	PTEN	SOS2	<b><i>GAPDH</i></b>
<b>C</b>	ACTB	BCAR1	FGFR2	GNA12	ITPR1	MAP2K2	MAPK9	PIK3C2A	PRKCD	PTK2	SRF	<b><i>LDHA</i></b>
<b>D</b>	ACTG1	CASP9	FGFR3	GNA13	ITPR2	MAP2K7	MEF2A	PIK3R2	PRKCE	PXN	TIMP3	<b><i>NONO</i></b>
<b>E</b>	ACTG2	CRK	FGFR4	GRB2	JUN	MAPK1	MYL9	PIK3R4	PRKCG	RAF1	VEGFA	<b><i>PPIH</i></b>
<b>F</b>	AKT1	CSK	FGFRL1	HRAS	KDR	MAPK10	NOS3	PLA2G4A	PRKCH	ROCK1	VEGFB	<b><i>GDC</i></b>
<b>G</b>	AKT2	EGF	FLT1	ITGAV	KIT	MAPK12	NRAS	PLCG1	PRKCI	RRAS	VEGFC	<b><i>PPC</i></b>
<b>H</b>	AKT3	ELK1	FLT4	ITGB1	KRAS	MAPK13	NRP1	PLCG2	PRKCQ	SHC1	VEGFD	<b><i>NTC</i></b>

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

**Appendix. Plate type choice chart.**

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**Plate type A**

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
ABI / Life Tech	ABI 5700	GK088-A
	ABI 7000	GK088-A
	ABI 7300	GK088-A
	ABI 7500	GK088-A
	ABI 7700	GK088-A
	ABI 7900 HT	GK088-A
	QuantStudio	GK088-A
	ViiA 7	GK088-A
Bio-Rad	Chromo4	GK088-A
	iCycler	GK088-A
	iQ5	GK088-A
	MyiQ	GK088-A
	MyiQ2	GK088-A
Eppendorf / Life Tech	Matercyler ep realplex 2	GK088-A
	Matercyler ep realplex 4	GK088-A
Stratagene	MX3000P	GK088-A
	MX3005P	GK088-A

**Plate type B**

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
ABI / Life Tech	ABI 7500 Fast	GK088-B
	ABI 7900 HT Fast	GK088-B
	QuantStudio Fast	GK088-B
	StepOnePlus	GK088-B
	ViiA 7 Fast	GK088-B
Bio-Rad	CFX Connect	GK088-B
	CFX96	GK088-B
	DNA Engine Opticon 2	GK088-B
Stratagene	MX4000	GK088-B

**Plate type C**

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
Roche	Lightcycler 96	GK088-C
	Lightcycler 480 (96-well)	GK088-C