



**GeneQuery™ Human Hippo Signaling Pathway qPCR Array Kit  
(GQH-HIP)  
Catalog #GK148**

**Product Description**

ScienCell's GeneQuery™ Human Hippo Signaling Pathway qPCR Array Kit (GQH-HIP) is designed to profile 88 key genes involved in the Hippo Signaling Pathway. The Hippo signaling pathway is crucial for regulating cell proliferation, apoptosis, and stem cell self-renewal. It plays a pivotal role in controlling cellular processes through a kinase cascade that ultimately modulates the activity of transcription factors. This pathway influences gene expression patterns and cellular outcomes, including growth suppression and tissue homeostasis. ScienCell's GeneQuery™ Human Hippo Signaling Pathway qPCR Array Kit provides a comprehensive platform for investigating the expression profiles of key genes involved in Hippo signaling. Brief examples of how included genes may be grouped according to function are shown below:

- Receptor: FZD1, FZD2, FZD3, FZD4, FZD5, FZD6
- Regulatory Element: YAP1, WWTR1, CCND1, ID2
- Transcription factor: MYC, SMAD2, TP73

**Note:** all gene names follow their official symbols by the Human Genome Organization Gene Nomenclature Committee (HGNC)

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<sup>2+</sup>, and no DMSO); (ii) the primer set recognizes all known transcript variants of the 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.

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- The No Template Control (NTC) is strongly recommended, and can be used to monitor the DNA contamination introduced during the workflows such as reagents, tips, and the lab bench.

### Kit Components

Component	Cat #	Quantity	Storage
GeneQuery™ array plate with lyophilized primers	GK148	1	4°C or -20°C
Optical PCR plate seal	NA	1	RT
Nuclease-free H <sub>2</sub> O	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-HIP 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 product should be stored at 4°C and is good for up to 12 months. For long-term storage (>1 year), store the product 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:

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

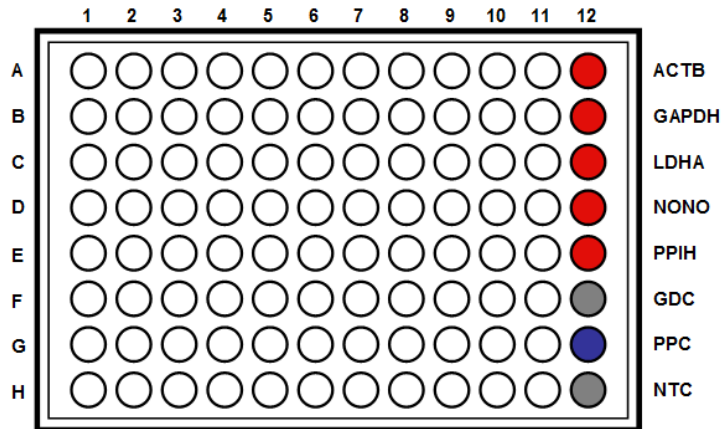


Table 2. Interpretation of control results:

<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 ≥ 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.)

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

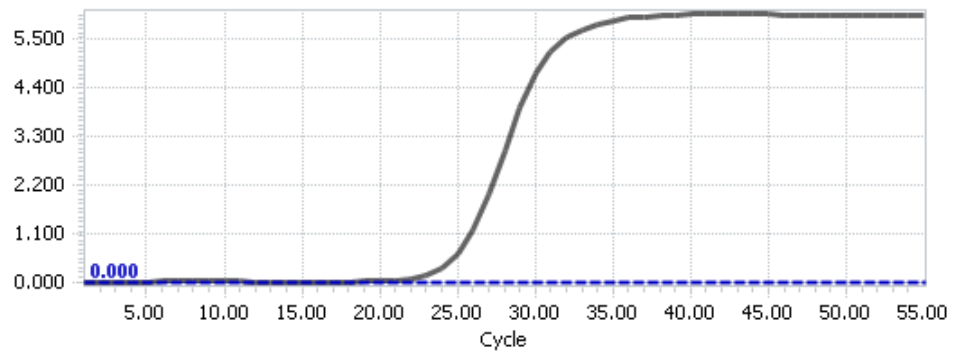
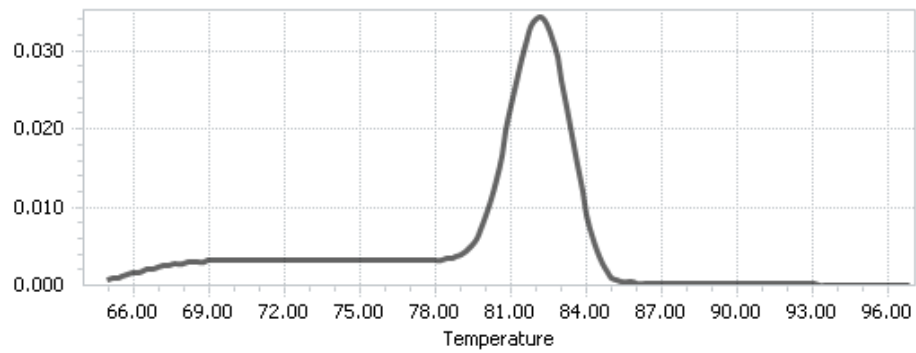


Figure 3. A typical melting peak of a qPCR product.



## **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 provide 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.

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

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

**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),  
 $\Delta Cq$  (GOI) =  $Cq$  (GOI, experimental sample) –  $Cq$  (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	ACTB	GAPDH	LDHA	NONO	PPIH
<b>Experimental</b>	21.61	22.19	17.16	17.84	20.12	19.64	26.40
<b>Control</b>	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™ qPCR Array Plate Layout\*  
 (8 *controls* in Bold and Italic)

*Note:* all gene names follow their official symbols by HGNC

	1	2	3	4	5	6	7	8	9	10	11	12
A	ACTG1	BIRC3	CRB1	DCHS2	FAT2	FZD4	HIPK2	MARK3	RASSF1	STK3	WWTR1	<b><i>ACTB</i></b>
B	AFP	BIRC5	CRB2	DLG1	FAT3	FZD5	ID2	MYC	RHOA	STK4	YAP1	<b><i>GAPDH</i></b>
C	AMOTL1	BMP2	CRB3	DLG2	FAT4	FZD6	ITGB2	NDRG1	SAV1	TAFFAZIN	YWHAB	<b><i>LDHA</i></b>
D	AMOTL2	BMP4	CSNK1D	DLG4	FRMD6	FZD7	LATS1	NF2	SERPINE1	TEAD3	YWHAE	<b><i>NONO</i></b>
E	APC	BMP5	CSNK1E	DLG5	FZD1	FZD8	LATS2	PPP1CA	SMAD2	TJP1	YWHAG	<b><i>PPIH</i></b>
F	AXIN1	CASP3	CTNNA1	DVL1	FZD10	FZD9	LLGL1	PPP2CA	SMAD7	TP53	YWHAH	<b><i>GDC</i></b>
G	BBC3	CCND1	CXCL10	DVL2	FZD2	GNA11	MAP4K3	PPP2CB	SNAI2	TP73	YWHAQ	<b><i>PPC</i></b>
H	BIRC2	CCND3	DCHS1	FAT1	FZD3	GNAQ	MAPK14	PRKAA1	STK11	WWC1	YWHAZ	<b><i>NTC</i></b>

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