



## **GeneQuery™ Human JNK Signaling Pathway qPCR Array Kit (GQ-JNK)**

Catalog #GK147

### **Product Description**

ScienCell's GeneQuery™ Human JNK Signaling Pathway qPCR array kit (GQ-JNK) is designed to profile 88 key genes involved in JNK Signaling Pathway. The JNK signaling pathway is a key component of the mitogen-activated protein kinase (MAPK) signaling pathway. It regulates numerous cellular processes, such as the cell cycle, differentiation, survival, and apoptosis. Upon activation, JNK triggers a cascade of molecular events that ultimately leads to the modulation of various transcription factors, gene expression patterns, and cellular outcomes. ScienCell's GeneQuery™ Human JNK Signaling Pathway qPCR Array Kit offers a comprehensive platform for investigating the expression profiles of key genes involved in JNK signaling. Brief examples of how included genes may be grouped according to function are shown below:

- Receptors: CCR5, CD44, TRAF2, BDKRB1, CELSR1, CALCRL, GRB2, IRS1
- Regulatory Elements: JUN, MECOM, PRDM16, RUNX2, SMAD4, ATF2, BATF3
- Cell Cycle Regulation: CDC25A ,CDC25B,CCND1,CCNE2

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 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) 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	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	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**

GQ-JNK 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 µl 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 µl
Nuclease-free H <sub>2</sub> O	variable
<b><i>Total volume</i></b>	<b><i>20 µl</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

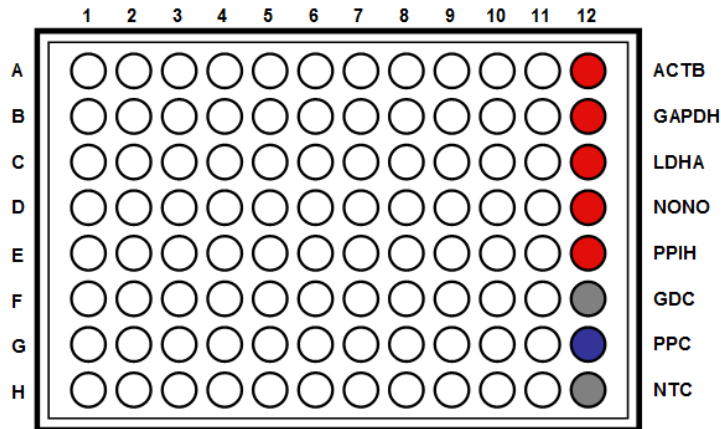


Table 2. Interpretation of control results:

<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.

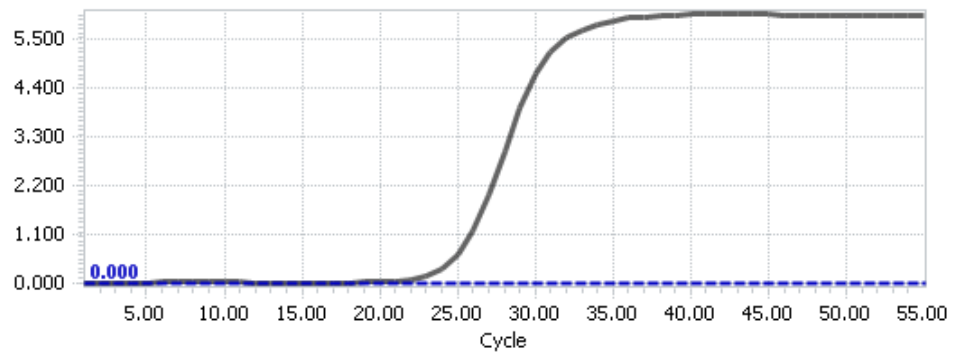
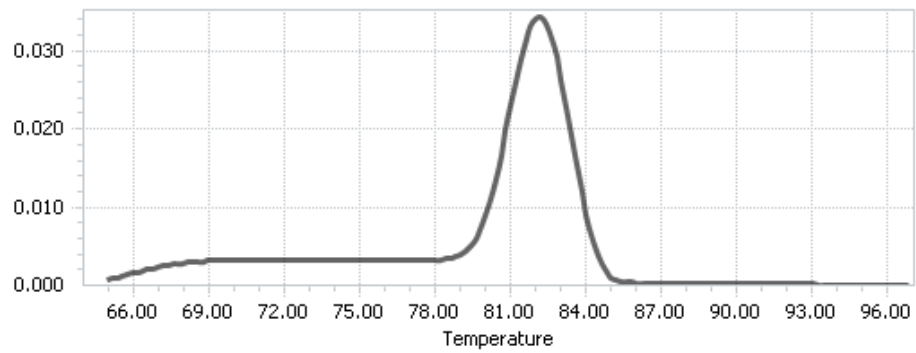


Figure 3. A typical melting peak of a qPCR product.



## **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**

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)

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	ADH7	BIRC2	CD79A	CHEK1	DAXX	HSF1	MAP1B	MAPK9	OXTR	RAC1	SOS1	<b><i>ACTB</i></b>
<b>B</b>	APAF1	CALCRL	CDC20	CLIP4	E2F1	IL2RB	MAP2	MCM4	PGK1	RHOA	SPIRE1	<b><i>GAPDH</i></b>
<b>C</b>	ATF2	CASP9	CDC25A	CNTN1	E2F4	IRS1	MAP3K10	MCM7	PIK3CA	RUNX2	SPP1	<b><i>LDHA</i></b>
<b>D</b>	BAD	CCND1	CDC25B	COX6A1	ELK1	ITCH	MAP3K11	MECOM	PKMYT1	SFTPD	STAT4	<b><i>NONO</i></b>
<b>E</b>	BATF3	CCNE2	CDK2	CRKL	FGFBP1	JUN	MAP3K7	NDUFB9	PLAT	SLC2A1	TCF7	<b><i>PPIH</i></b>
<b>F</b>	BCL2	CCR5	CDKN2C	CUL1	FGL2	KIF20A	MAP4K3	NFATC2	PLK1	SLC2A10	TP53	<b><i>GDC</i></b>
<b>G</b>	BDKRB1	CD44	CELSR1	CYP11A1	GRB2	KRT16	MAPK10	NTS	PRDM16	SMAD4	TRADD	<b><i>PPC</i></b>
<b>H</b>	BID	CD69	CENPA	CYP19A1	HOMER1	LDB1	MAPK8	ORC6	PXN	SMC3	TRAF2	<b><i>NTC</i></b>