



**GeneQuery™ Human Axon Guidance qPCR Array Kit**  
**(GQH-AXN)**  
Catalog #GK084

**Product Description**

ScienCell's GeneQuery™ Human Axon Guidance qPCR Array (GQH-AXN) profiles 88 key genes involved in neural pathfinding. During neural development, neurons send projections otherwise known as axons to reach targets and transmit electric signals. Axons follow specific paths and the underlying pathfinding mechanism is an area of growing research. Below are brief examples of how included genes may be grouped according to their signaling pathways or protein classes:

- **Ephrins:** EFNA1, EFNA5, EFNB3, EPHA1, EPHB1
- **Semaphorins:** SEMA3A, SEMA4D, SEMA5A, SEMA6B, SEMA7A
- **WNT signaling:** WNT4, WNT5A, SHH, SMO, FZD3
- **Netrin signaling:** NTN1, NTN4, NTNG1, NTNG2, DCC, UNC5A
- **Cell adhesion molecules:** CAMK2A, CAMK2B, CAMK2D, CAMK2G

**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 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 workflow such as reagents, tips, and the lab bench.

**Kit Components**

Component	Cat #	Quantity	Storage
GeneQuery™ array plate with lyophilized primers	GK084	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)**

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-AXN 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

**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

cDNA template	0.2 – 250 ng
2x qPCR master mix	10 µl
Nuclease-free H <sub>2</sub> O	variable
<b>Total volume</b>	<b>20 µl</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.

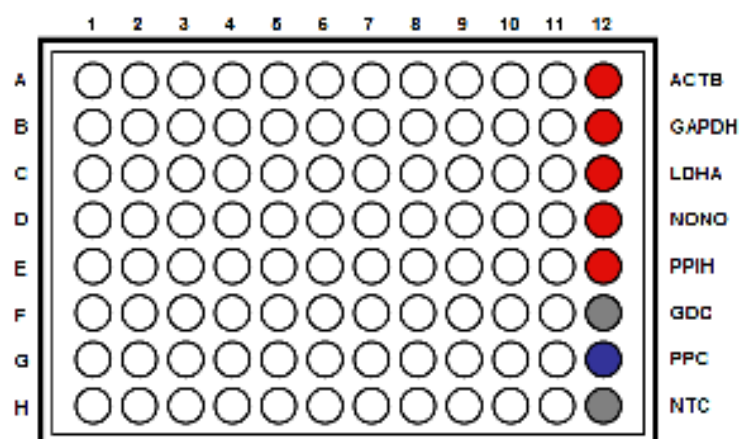


Table 2. Interpretation of control results:

<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.)

Rev.1

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

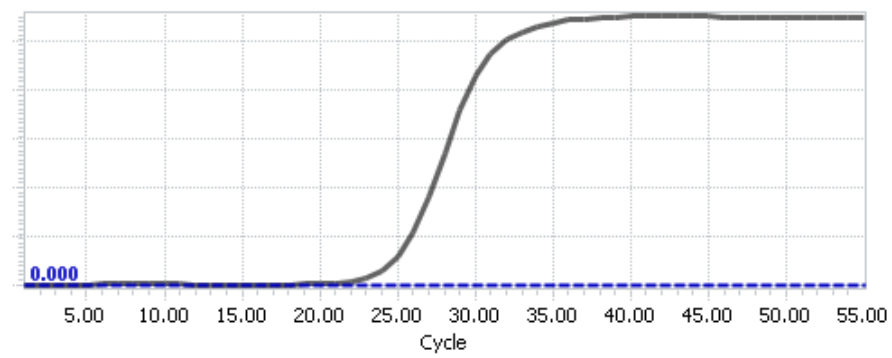
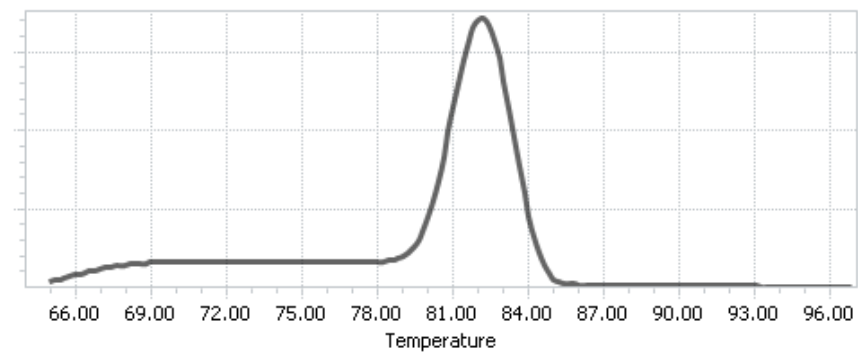


Figure 3. A typical melting peak of a qPCR product.



## **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:**  $\Delta Cq \text{ (HKG)} = Cq \text{ (HKG, experimental sample)} - Cq \text{ (HKG, control sample)}$ , 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**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 Axon Guidance 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
<b>A</b>	ABL1	CAMK2B	EFNA1	EPHA1	EPHB3	NRP1	RAC1	SEMA3D	SEMA4F	SLIT2	TRPC6	<b><i>ACTB</i></b>
<b>B</b>	ABLIM3	CAMK2D	EFNA2	EPHA2	EPHB4	NRP2	RAF1	SEMA3E	SEMA4G	SLIT3	UNC5A	<b><i>GAPDH</i></b>
<b>C</b>	ARHGEF12	CAMK2G	EFNA3	EPHA3	F2RL2	NTN1	ROBO1	SEMA3F	SEMA5A	SMO	UNC5B	<b><i>LDHA</i></b>
<b>D</b>	BMP7	CFL1	EFNA4	EPHA4	FES	NTN4	ROBO2	SEMA3G	SEMA6B	TRPC1	UNC5C	<b><i>NONO</i></b>
<b>E</b>	BMPR1B	CFL2	EFNA5	EPHA5	FYN	NTNG1	ROCK1	SEMA4A	SEMA6D	TRPC1	UNC5D	<b><i>PPIH</i></b>
<b>F</b>	BMPR2	CXCR4	EFNB1	EPHA7	FZD3	NTNG2	RYK	SEMA4B	SEMA7A	TRPC3	WNT4	<b><i>GDC</i></b>
<b>G</b>	BOC	DCC	EFNB2	EPHB1	NCK1	PAK2	SEMA3A	SEMA4C	SHH	TRPC4	WNT5A	<b><i>PPC</i></b>
<b>H</b>	CAMK2A	DPYSL2	EFNB3	EPHB2	NFATC2	PAK4	SEMA3C	SEMA4D	SLIT1	TRPC5	WNT5B	<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	GK084-A
	ABI 7000	GK084-A
	ABI 7300	GK084-A
	ABI 7500	GK084-A
	ABI 7700	GK084-A
	ABI 7900 HT	GK084-A
	QuantStudio	GK084-A
	ViiA 7	GK084-A
Bio-Rad	Chromo4	GK084-A
	iCycler	GK084-A
	iQ5	GK084-A
	MyiQ	GK084-A
	MyiQ2	GK084-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK084-A
	Matercycler ep realplex 4	GK084-A
Stratagene	MX3000P	GK084-A
	MX3005P	GK084-A

**Plate type B**

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
ABI / Life Tech	ABI 7500 Fast	GK084-B
	ABI 7900 HT Fast	GK084-B
	QuantStudio Fast	GK084-B
	StepOnePlus	GK084-B
	ViiA 7 Fast	GK084-B
Bio-Rad	CFX Connect	GK084-B
	CFX96	GK084-B
	DNA Engine Opticon 2	GK084-B
Stratagene	MX4000	GK084-B

**Plate type C**

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
Roche	Lightcycler 96	GK084-C
	Lightcycler 480 (96-well)	GK084-C