



## GeneQuery™ Human Astrocyte Cell Biology qPCR Array Kit (GQH-AST) Catalog #GK009

### Product Description

ScienCell's GeneQuery™ Human Astrocyte Cell Biology qPCR array kit (GQH-AST) is designed to facilitate gene expression profiling of key genes involved in (i) human astrocyte development and maintenance; (ii) genes that are much up-regulated in astrocytes; and (iii) related signaling pathways, such as Notch, TGFB, JAK/STAT, and MAPK pathways. 88 genes are selected in this kit based on database and literature research.

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 ( $\beta$ -actin, 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	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 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-AST 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

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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.

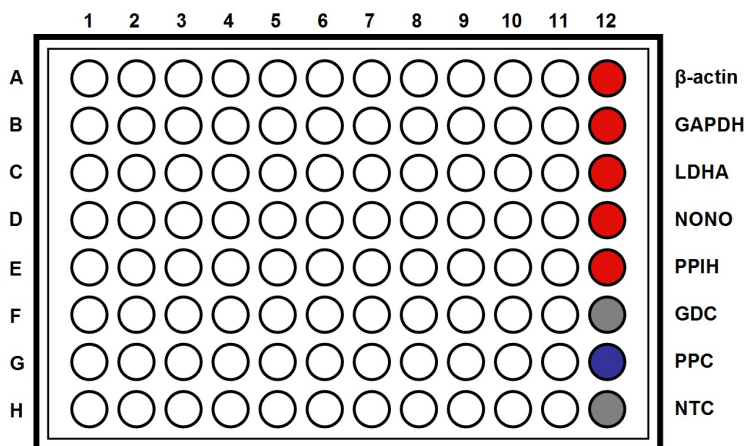


Table 2. Interpretation of control results:

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

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

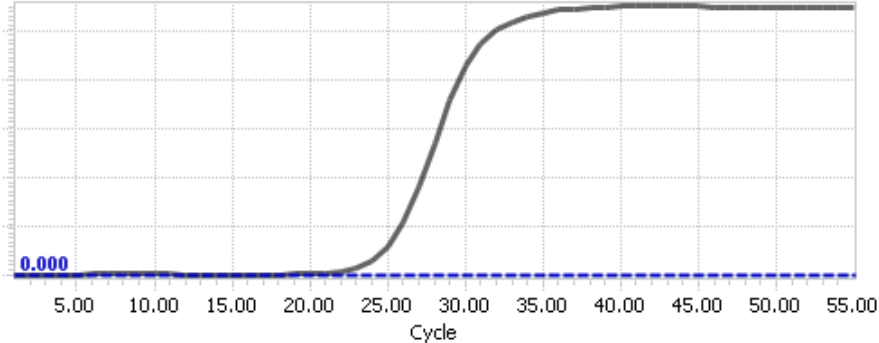
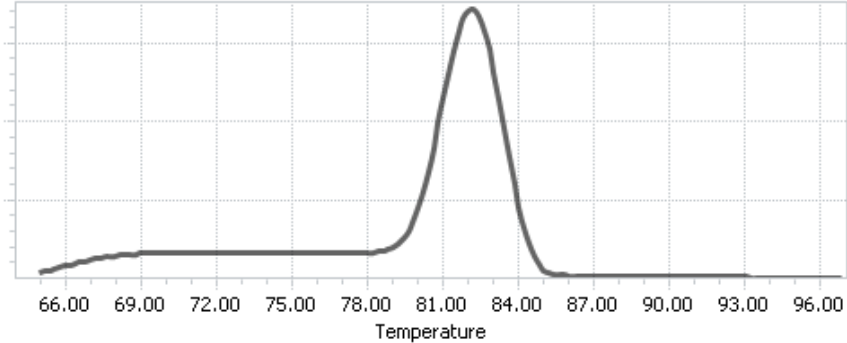


Figure 3. A typical melting peak of a qPCR product.



## **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 Astrocyte Cell Biology 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>	AFF2	CD44	CRYAB	EPM2A	HEY1	ITGA3	LMO2	NHLRC1	POMC	SCN1B	TGFB2	<i><b>β-actin</b></i>
<b>B</b>	ATRX	CDKL5	DLK1	FMR1	HOPX	JAG1	LPPR4	NOTCH1	PRDM16	SLC25A18	TGFB3	<i><b>GAPDH</b></i>
<b>C</b>	BEND6	CDKN2B	DNER	FOXG1	HTT	JAK1	MAPK10	NOTCH3	PRRX1	SMAD2	TGFBR3	<i><b>LDHA</b></i>
<b>D</b>	BHLHE40	CHRNA4	DPF3	FXR1	IGFBP7	KLF5	MAPK14	NR2E1	PTPRE	SMAD3	TH	<i><b>NONO</b></i>
<b>E</b>	BHLHE41	CLN8	DUSP1	FXR2	IL1RAPL1	LEP	MECP2	NUFIP1	RBPJ	STAT2	TM4SF1	<i><b>PPIH</b></i>
<b>F</b>	BMP7	CNTF	DUSP18	GABRG2	IL2RA	LGALS3	MVP	NUFIP2	RPS27A	STAT6	TSPAN7	<i><b>GDC</b></i>
<b>G</b>	CACNA1H	CNTFR	EFHC1	GFAP	IL6	LHX2	NFIC	OTX1	S100A6	SYNC	UBE3A	<i><b>PPC</b></i>
<b>H</b>	CASP3	COBL	EMX2	GLIPR1	IRS2	LIF	NFIX	PHF11	SCN1A	TDRD7	ZFP3	<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

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
ABI / Life Tech	ABI 5700	GK009-A
	ABI 7000	GK009-A
	ABI 7300	GK009-A
	ABI 7500	GK009-A
	ABI 7700	GK009-A
	ABI 7900 HT	GK009-A
	QuantStudio	GK009-A
	ViiA 7	GK009-A
Bio-Rad	Chromo4	GK009-A
	iCycler	GK009-A
	iQ5	GK009-A
	MyiQ	GK009-A
	MyiQ2	GK009-A
Eppendorf / Life Tech	Matercyler ep realplex 2	GK009-A
	Matercyler ep realplex 4	GK009-A
Stratagene	MX3000P	GK009-A
	MX3005P	GK009-A

### Plate type B

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
ABI / Life Tech	ABI 7500 Fast	GK009-B
	ABI 7900 HT Fast	GK009-B
	QuantStudio Fast	GK009-B
	StepOnePlus	GK009-B
	ViiA 7 Fast	GK009-B
Bio-Rad	CFX Connect	GK009-B
	CFX96	GK009-B
	DNA Engine Opticon 2	GK009-B
Stratagene	MX4000	GK009-B

### Plate type C

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
Roche	Lightcycler 96	GK009-C
	Lightcycler 480 (96-well)	GK009-C