![ScienCell Research Laboratories logo]

GeneQuery™ Human Neural Plasticity qPCR Array Kit (GQH-NPL)
Catalog #GK007

Product Description
ScienCell's GeneQuery™ Human Neural Plasticity qPCR array kit (GQH-NPL) is designed to facilitate gene expression profiling of key genes involved in human synaptic plasticity and diseases, such as attention deficit/hyperactivity disorder (ADHD), autism, Down syndrome, and schizophrenia. 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^{2+}, 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 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.
- 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	GK007	1	4°C or -20°C
Optical PCR plate seal	N/A	1	RT
Nuclease-free H_2O	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-NPL 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$_2$O	variable
Total volume	*20 µl*

   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$_2$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 H2O 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	
Annealing	65°C	20 sec	40
Extension	72°C	20 sec	
Data acquisition	Plate read		
Recommended	*Melting curve analysis*		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.
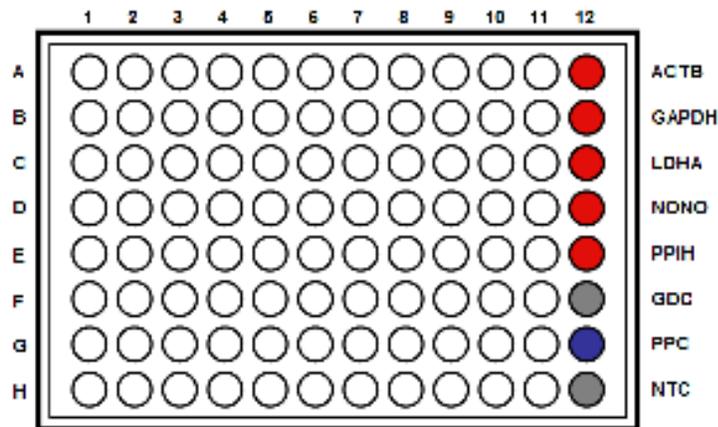
Table 2. Interpretation of control results:

Controls	Results	Interpretation	Suggestions
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.
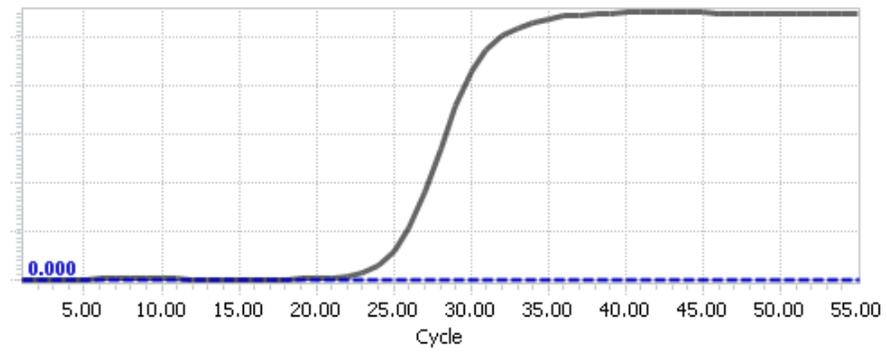

Figure 3. A typical melting peak of a qPCR product.

Rev. 1
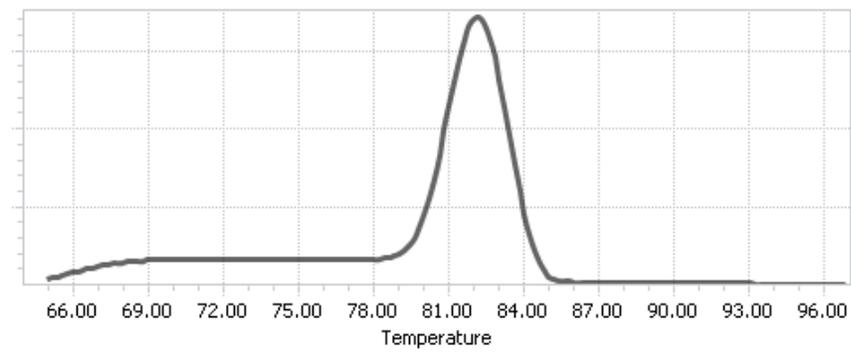
Quantification Method: Comparative ΔΔCq (Quantification Cycle Value) Method

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, ΔCq (ref) is the quantification cycle number change for that housekeeping gene (HKG) between an experimental sample and control sample.

   $$\Delta Cq \ (ref) = Cq \ (HKG, \text{experimental sample}) - Cq \ (HKG, \text{control sample})$$

   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 ΔCq of the selected housekeeping genes.

   $$\Delta Cq \ (ref) = \text{average} \ (\Delta Cq \ (HKG1), \Delta Cq \ (HKG2),......., \Delta Cq \ (HKG \ n))$$ (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:

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

   Note: ΔCq (HKG) = Cq (HKG, experimental sample) - Cq (HKG, control sample), and ΔCq (HKG) value can be positive, 0, or negative.

3. For any of your genes of interest (GOI),

   $$\Delta Cq \ (GOI) = Cq \ (GOI, \text{experimental sample}) - Cq \ (GOI, \text{control sample})$$

   $$\Delta\Delta Cq = \Delta Cq \ (GOI) - \Delta Cq \ (ref)$$

   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 ΔΔCq (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.

	Genes of Interest		Housekeeping Genes				
Samples	GOI1	GOI2	*ACTB*	*GAPDH*	*LDHA*	*NONO*	*PPIH*
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

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

$$\Delta Cq\ (GOI1) = 21.61 - 33.13$$
$$= -11.52$$

$$\Delta Cq\ (GOI2) = 22.19 - 26.47$$
$$= -4.28$$

$$\Delta\Delta Cq\ (GOI1) = \Delta Cq\ (GOI1) - \Delta Cq\ (ref)$$
$$= -11.52 - (-0.43)$$
$$= -11.09$$

$$\Delta\Delta Cq\ (GOI2) = \Delta Cq\ (GOI2) - \Delta Cq\ (ref)$$
$$= -4.28 - (-0.43)$$
$$= -3.85$$

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

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

Conclusion: Upon treatment, expression level of GOI1 increased 2,180 fold, and expression level of GOI2 increased 14.4 fold.

ScienCell™
Research Laboratories

**GeneQuery™ Human Neural Plasticity qPCR Array Kit
(GQH-NPL)**
Catalog #GK007

GeneQuery™ Human Neural Plasticity qPCR Array Plate Layout* (*8 controls* in Bold and Italic)

	1	2	3	4	5	6	7	8	9	10	11	12
A	ADAM10	CDH2	DISC2	FBN1	GRIA4	HTR2A	MAPK1	NLGN4X	PCDH8	PPP2CA	RHEB	*ACTB*
B	ADCY1	CEBPB	DSCAM	FGFR2	GRIN2B	IGF1	MECP2	NOS1	PICK1	PRKCA	RPL10	*GAPDH*
C	ADCY8	CEBPD	DSCR3	FOS	GRIN2D	INHBA	MMP9	NPTX2	PIGP	PRKG1	SLC6A2	*LDHA*
D	ARC	CNR1	EGR1	GABRA5	GRIP1	JUN	NFKB1	NR4A1	PIM1	RAB3A	SLC6A4	*NONO*
E	AUTS2	CNTNAP2	EGR2	GABRB3	GRM2	JUNB	NFKBIB	NRG1	PLAT	RELA	SRF	*PPIH*
F	BDNF	COMT	EGR4	GNAI1	GRM3	KCNQ1	NGF	NTF3	PLCG1	RELN	SYNPO	*GDC*
G	CAMK2A	CREB1	EN2	GRIA1	HOMER1	KLF10	NGFR	NTF4	PPP1CA	RGS2	TNF	*PPC*
H	CAMK2G	DAOA	EPHB2	GRIA2	HTR1B	MAOA	NLGN3	NTRK2	PPP1CC	RGS4	YWHAQ	*NTC*

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

Appendix. Plate type choice chart.

Plate type A

Brand	Model	kit catalog #
ABI / Life Tech	ABI 5700	GK007-A
	ABI 7000	GK007-A
	ABI 7300	GK007-A
	ABI 7500	GK007-A
	ABI 7700	GK007-A
	ABI 7900 HT	GK007-A
	QuantStudio	GK007-A
	ViiA 7	GK007-A
Bio-Rad	Chromo4	GK007-A
	iCycler	GK007-A
	iQ5	GK007-A
	MyiQ	GK007-A
	MyiQ2	GK007-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK007-A
	Matercycler ep realplex 4	GK007-A
Stratagene	MX3000P	GK007-A
	MX3005P	GK007-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK007-B
	ABI 7900 HT Fast	GK007-B
	QuantStudio Fast	GK007-B
	StepOnePlus	GK007-B
	ViiA 7 Fast	GK007-B
Bio-Rad	CFX Connect	GK007-B
	CFX96	GK007-B
	DNA Engine Opticon 2	GK007-B
Stratagene	MX4000	GK007-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK007-C
	Lightcycler 480 (96-well)	GK007-C