Rev.1

GeneQuery™ Human Ephrin Signaling qPCR Array Kit (GQH-EPH)

Catalog #GK089

Product Description

ScienCell's GeneQuery™ Human Ephrin Signaling qPCR Array Kit (GQH-EPH) profiles 88 key genes involved in ephrin signaling and signal transduction. Ephrin signaling is involved in such developmental processes as axonal guidance, angiogenesis, cell migration, embryonic development, and segmentation. Ephrin ligands and receptors are subdivided into two classes based on their structure and binding affinities: ephrin-A and ephrin-B. A more unique aspect of ephrin signaling is that it can occur bidirectionally expressing as both a "forward signal" and a "reverse signal." Brief examples of how included genes may be grouped according to their function are shown below:

- **Signaling receptors:** EPHA2, EPHA4, EPHB1, EPHB2, EPHB4
- **Ligands:** EFNA1, EFNA2, EFNA5, EFNB1, EFNB2
- **Cytoskeletal dynamics:** ACTB, ACTG1, ACTN1, CFL1, DNM1
- **Vesicle Trafficking:** AP2B1, AP2S1, APH1A, CAV1, CLTA
- **Hemostasis:** FN1, FYN, ITGAVM ITGB1, LYN

GeneQuery™ qPCR array kits are qPCR ready in a 96-well plate format, with each well containing one primer set that recognizes and efficiently amplifies a specific 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 the target gene, unless otherwise noted; 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), which detects gDNA contamination in cDNA samples. This primer set targets a non-transcribed region of the genome.
- Positive PCR Control (PPC), which 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), which can be used to monitor DNA contamination introduced during workflow (e.g. from such sources as reagents, tips, and the lab bench).

Kit Components

Component	Cat #	Quantity	Storage
GeneQuery™ array plate with lyophilized primers	GK089	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 primer sets are validated by qPCR with melt curve analysis and analyzed by gel electrophoresis. Single band amplification is confirmed for each set of primers.

Product Use

GQH-EPH 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

This 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 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_2O	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_2O 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	40
Annealing	65°C	20 sec	
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.

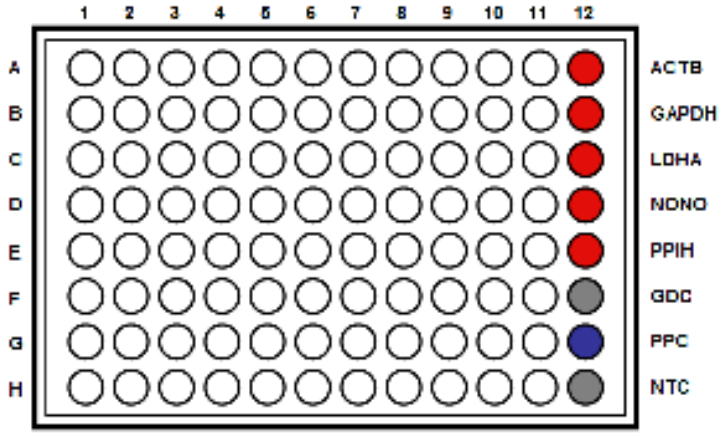


Table 2. Interpretation of control results:

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 ≥ 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.

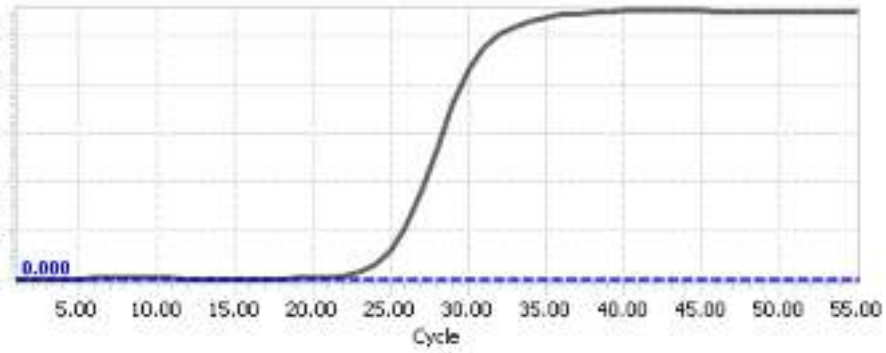
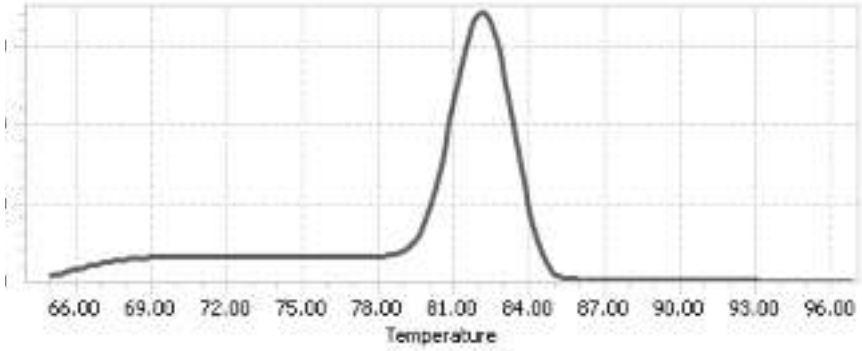


Figure 3. A typical melting peak of a qPCR product.



Quantification Method: Comparative 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.

   ΔCq (ref) = Cq (HKG, experimental sample) - Cq (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 ΔCq of the selected housekeeping genes.

   ΔCq (ref) = average (ΔCq (HKG1), ΔCq (HKG2),....., ΔCq (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:

   ΔCq (ref) = (ΔCq(ACTB)+ΔCq(GAPDH)+ΔCq(LDHA)+ΔCq(NONO)+ΔCq(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),

   ΔCq (GOI) = Cq (GOI, experimental sample) - Cq (GOI, control sample)

   ΔΔCq = ΔCq (GOI) - ΔCq (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

ΔCq (ref) = (ΔCq(ACTB)+ΔCq(GAPDH)+ΔCq(LDHA)+ΔCq(NONO)+ΔCq(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

ΔCq (GOI1) = 21.61 - 33.13
= -11.52

ΔCq (GOI2) = 22.19 - 26.47
= -4.28

ΔΔCq (GOI1) = ΔCq (GOI1) - ΔCq (ref)
= -11.52 - (-0.43)
= -11.09

ΔΔCq (GOI2) = ΔCq (GOI2) - ΔCq (ref)
= -4.28 - (-0.43)
= -3.85

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

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.

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	ACTB	AP2S1	DNM1	EFNB3	EPHB2	GRIA1	LIMK1	MYL6	PIK3CG	RAP1A	TF	***ACTB***
B	ACTG1	APH1A	EFNA1	EPHA1	EPHB3	GRIN1	LYN	MYL9	PIK3R1	RASA1	TIAM1	***GAPDH***
C	ACTN1	CAV1	EFNA2	EPHA2	EPHB4	GRIN2B	MAP2K1	NCK1	PSEN2	RGS3	TLN1	***LDHA***
D	ADAM10	CDC42	EFNA3	EPHA3	FN1	HRAS	MAPK1	NCK2	PSENEN	RHOA	VAV2	***NONO***
E	AP2A1	CFL1	EFNA4	EPHA4	FYN	ITGA5	MMP2	NCSTN	PTK2	ROCK1	VAV3	***PPIH***
F	AP2A2	CLTA	EFNA5	EPHA5	GNAI1	ITGAV	MMP9	PAK1	PXN	SDC2	VCL	***GDC***
G	AP2B1	CLTB	EFNB1	EPHA7	GRB2	ITGB1	MYH10	PAK2	RAC1	SRC	VEGFA	***PPC***
H	AP2M1	CLTC	EFNB2	EPHB1	GRB7	KDR	MYH9	PIK3CA	RAF1	SYNJ1	WASL	***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	GK089-A
	ABI 7000	GK089-A
	ABI 7300	GK089-A
	ABI 7500	GK089-A
	ABI 7700	GK089-A
	ABI 7900 HT	GK089-A
	QuantStudio	GK089-A
	ViiA 7	GK089-A
Bio-Rad	Chromo4	GK089-A
	iCycler	GK089-A
	iQ5	GK089-A
	MyiQ	GK089-A
	MyiQ2	GK089-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK089-A
	Matercycler ep realplex 4	GK089-A
Stratagene	MX3000P	GK089-A
	MX3005P	GK089-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK089-B
	ABI 7900 HT Fast	GK089-B
	QuantStudio Fast	GK089-B
	StepOnePlus	GK089-B
	ViiA 7 Fast	GK089-B
Bio-Rad	CFX Connect	GK089-B
	CFX96	GK089-B
	DNA Engine Opticon 2	GK089-B
Stratagene	MX4000	GK089-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK089-C
	Lightcycler 480 (96-well)	GK089-C