



## GeneQuery™ Human Endocytosis qPCR Array Kit (GQH-ECY) Catalog #GK022

### Product Description

ScienCell's GeneQuery™ Human Endocytosis qPCR Array Kit (GQH-ECY) is designed to facilitate gene expression profiling of 88 key genes involved in cellular endocytosis. Endocytosis pathways are used to mediate signal transduction from the cell surface and to internalize or transport particulate matter. Brief examples of how included genes may be grouped according to endocytic pathway are shown below:

- **Clathrin-mediated:** CLTA, CLINT1, AP2A1, SNX9, EPN1
- **Caveolae:** CAV1, CAV2, CAV3, PTRF, FLOT1
- **Macropinocytosis:** KRAS, SRC, PAK1, PAK2, HDAC6
- **Phagocytosis and degradation:** IQGAP1, ROCK1, UBB, DCTN1, AMPH

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<sup>2+</sup> 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 ( $\beta$ -actin, 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 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), 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	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 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-ANG 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

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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
<i>Total volume</i>	<i>20 <math>\mu</math>l</i>

**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.

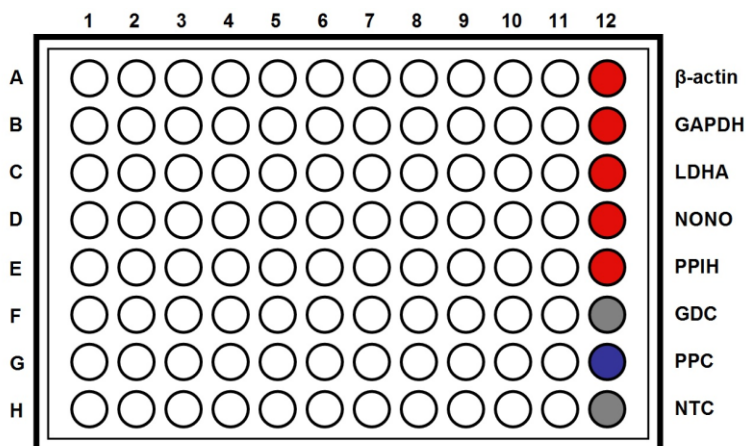


Table 2. Interpretation of control results:

<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.)

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

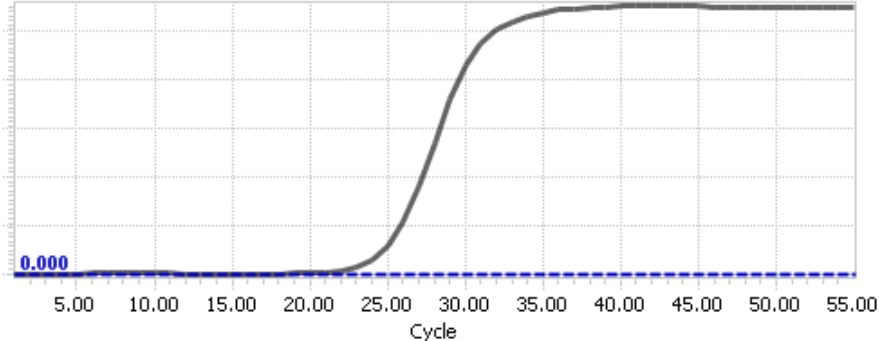
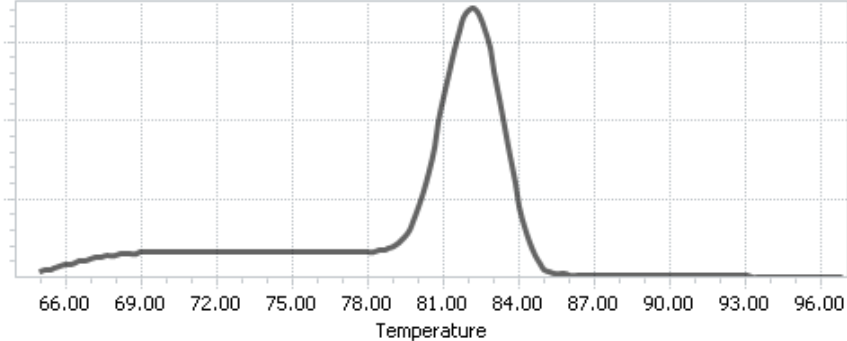


Figure 3. A typical melting peak of a qPCR product.



## 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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**(GQH-ECY)**  
 Catalog #GK022

GeneQuery™ Human Endocytosis 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>	ACTB	ARHGAP26	CLTB	DOCK1	EPN1	IQGAP1	MAPK8IP3	PIKFYVE	RAB11FIP5	RALBP1	SNX9	<i><b>β-actin</b></i>
<b>B</b>	AMPH	CAV1	CLTC	DYNAP	EPN2	KCNJ5	MYO5B	PLD2	RAB22A	RBSN	SPAG9	<i><b>GAPDH</b></i>
<b>C</b>	AP2A1	CAV2	CTTN	ECT2	EPN3	KLC1	MYO6	PRKCI	RAB35	REPS2	SRC	<i><b>LDHA</b></i>
<b>D</b>	AP2B1	CAV3	DCTN1	EEA1	EPS15	KLC2	MYOF	PTRF	RAB4A	RHOA	STX5	<i><b>NONO</b></i>
<b>E</b>	AP2S1	CDC42	DCTN2	EHD1	EXOC5	KRAS	PAK1	RAB10	RAB5A	ROCK1	SYNJ1	<i><b>PPIH</b></i>
<b>F</b>	ARF1	CEP164	DENND1A	EHD2	FLOT1	LAMP1	PAK2	RAB11A	RAB7A	SDCBP	UBB	<i><b>GDC</b></i>
<b>G</b>	ARF6	CLINT1	DNAL1	EHD3	FLOT2	MAPK1	PARD3	RAB11FIP2	RAB8A	SNX1	VPS45	<i><b>PPC</b></i>
<b>H</b>	ARHGAP10	CLTA	DNM1	EHD4	HDAC6	MAPK8IP2	PARD6A	RAB11FIP3	RACGAP1	SNX4	WWCI	<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	GK022-A
	ABI 7000	GK022-A
	ABI 7300	GK022-A
	ABI 7500	GK022-A
	ABI 7700	GK022-A
	ABI 7900 HT	GK022-A
	QuantStudio	GK022-A
	ViiA 7	GK022-A
Bio-Rad	Chromo4	GK022-A
	iCycler	GK022-A
	iQ5	GK022-A
	MyiQ	GK022-A
	MyiQ2	GK022-A
Eppendorf / Life Tech	Matercyler ep realplex 2	GK022-A
	Matercyler ep realplex 4	GK022-A
Stratagene	MX3000P	GK022-A
	MX3005P	GK022-A

### Plate type B

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
ABI / Life Tech	ABI 7500 Fast	GK022-B
	ABI 7900 HT Fast	GK022-B
	QuantStudio Fast	GK022-B
	StepOnePlus	GK022-B
	ViiA 7 Fast	GK022-B
Bio-Rad	CFX Connect	GK022-B
	CFX96	GK022-B
	DNA Engine Opticon 2	GK022-B
Stratagene	MX4000	GK022-B

### Plate type C

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
Roche	Lightcycler 96	GK022-C
	Lightcycler 480 (96-well)	GK022-C