



**GeneQuery™ Human Epithelial Cell Biology qPCR Array Kit
(GQH-EPI)
Catalog #GK005**

Product Description

ScienCell's GeneQuery™ human epithelial cell biology qPCR array kit (GQH-EPI) is designed to facilitate gene expression profiling of key genes involved in human epithelial cell biology. 88 genes are selected in this kit based on public 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 Mg²⁺, 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 (β-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 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	Quantity	Storage
GeneQuery™ array plate with lyophilized primers	1	4°C or -20°C
Optical PCR plate seal	1	RT
Nuclease-free H ₂ 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-EPI 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 μ l PCR reactions for one well as shown in Table 1.

Table 1

cDNA template	0.2 – 250 ng
2x qPCR master mix	10 μ l
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 H₂O 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		
<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.

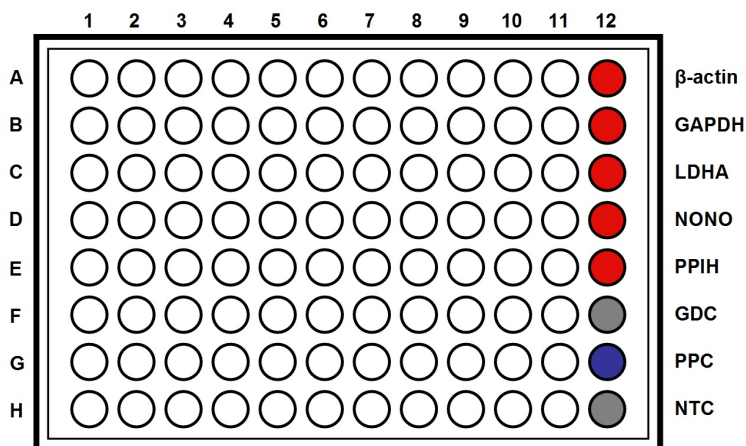


Table 2. Interpretation of control results:

<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.

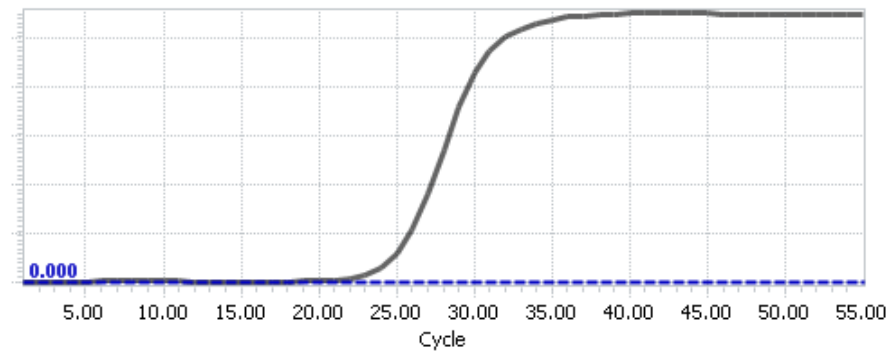
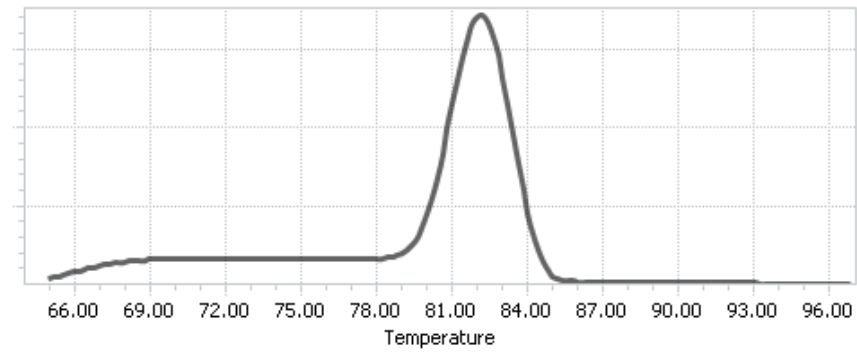


Figure 3. A typical melting peak of a qPCR product.





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GeneQuery™ Human Epithelial Cell Biology qPCR Array Plate Layout* (*8 controls* in Bold and Italic)

	1	2	3	4	5	6	7	8	9	10	11	12
A	APC	CDH2	DLL4	ERBB2	FZD5	HNF1A	LGR5	NEUROG3	PDX1	ROCK2	STAT1	<i>β-actin</i>
B	APOC2	CDX1	EEF1B2	FGF7	FZD7	HNF1B	MAX	NKX2B	PFDN2	RPL36	STK11	<i>GAPDH</i>
C	ATOH1	CDX2	EGFR	FGFR1	FZD8	IHH	MMP9	NOP58	PPARD	SHROOM3	TARS	<i>LDHA</i>
D	BLIMP1	CGN	EIF2AK3	FGFR2	GATA4	JAM1	MSI1	NOTCH1	PTK6	SKIV2L	TGFBR2	<i>NONO</i>
E	BMPR1A	CLDN1	ELF3	FGFR3	GATA6	JAM2	MXD1	NOTCH2	RB1	SNAI1	TJP1	<i>PPIH</i>
F	CALB2	CLDN2	EPHB2	FKBP4	GFI1	KLF4	MYBL2	OCLN	RBMS1	SOX9	VAV2	<i>GDC</i>
G	CBFA2T2	CTNNB1	EPHB3	FOXA1	GFM1	LDLR	MYC	PAX6	RBPJ	SPDEF	VIL2	<i>PPC</i>
H	CDH1	DDX56	EPRS	FOXA2	GSN	LGR4	NEUROD1	PCNA	ROCK1	SPRR2A	VIM	<i>NTC</i>

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