GeneQuery™ Human Cell Cycle qPCR Array Kit (GQH-CCY)

Catalog #GK003

Product Description

ScienCell's GeneQuery™ Human Cell Cycle qPCR array kit (GQH-CCY) is designed to facilitate gene expression profiling of key genes involved in human cell cycle progression. 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	Quantity	Storage
GeneQuery™ array plate with lyophilized primers	1	4°C or -20°C
Optical PCR plate seal	1	RT
Nuclease-free H_2O	2 mL	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-CCY 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_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.

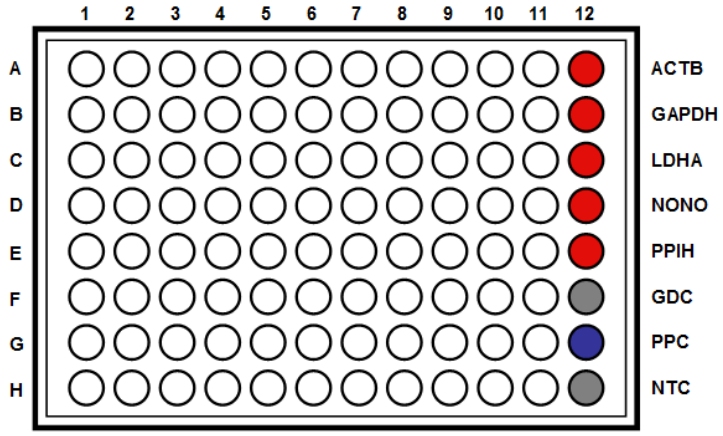


Table 2. Interpretation of control results:

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.

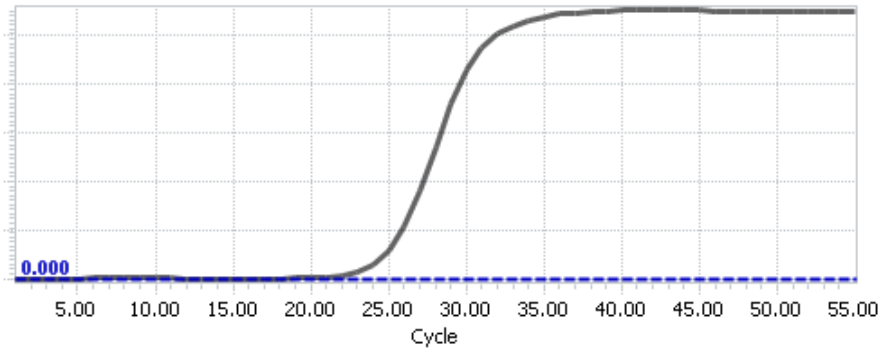
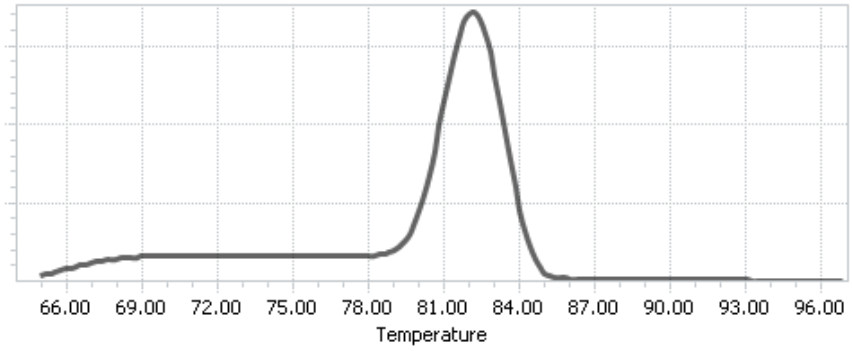


Figure 3. A typical melting peak of a qPCR product.



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.

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

   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.

   $$\Delta Cq\ (ref) = average\ (\Delta Cq\ (HKG1), \Delta Cq\ (HKG2), ..., \Delta Cq\ (HKG\ n))$$ (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),

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

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

   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

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

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

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

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

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

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.

GeneQuery™ Human Cell Cycle qPCR Array Kit (GQH-CCY)

Catalog #GK003

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	ANAPC2	BUB3	CCND2	CDC20	CDK6	CHEK1	E2F1	HDAC1	MCM3	RAD1	SMAD2	***ACTB***
B	ATM	CASP3	CCND3	CDC25A	CDK7	CHEK2	E2F2	HDAC2	MCM4	RAD17	SMC3	***GAPDH***
C	ATR	CCNA1	CCNE2	CDC25C	CDK8	CKS1B	E2F4	HUS1	MCM5	RAD51	STAG1	***LDHA***
D	AURKB	CCNA2	CCNF	CDC34	CDKN1A	CKS2	E2F5	KNTC1	MDM2	RAD9A	TFDP1	***NONO***
E	BCCIP	CCNB1	CCNG1	CDC6	CDKN1B	CUL1	EP300	KPNA2	MYC	RB1	TFDP2	***PPIH***
F	BIRC5	CCNB2	CCNH	CDK1	CDKN2A	CUL2	GADD45A	MAD2L1	NBN	RBL1	TGFB1	***GDC***
G	BRCA1	CCNC	CCNT1	CDK2	CDKN2B	CUL3	GSK3B	MAD2L2	ORC1	RBL2	TP53	***PPC***
H	BRCA2	CCND1	CDC16	CDK4	CDKN3	DBF4	GTSE1	MCM2	PCNA	SKP2	WEE1	***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	GK003-A
	ABI 7000	GK003-A
	ABI 7300	GK003-A
	ABI 7500	GK003-A
	ABI 7700	GK003-A
	ABI 7900 HT	GK003-A
	QuantStudio	GK003-A
	ViiA 7	GK003-A
Bio-Rad	Chromo4	GK003-A
	iCycler	GK003-A
	iQ5	GK003-A
	MyiQ	GK003-A
	MyiQ2	GK003-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK003-A
	Matercycler ep realplex 4	GK003-A
Stratagene	MX3000P	GK003-A
	MX3005P	GK003-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK003-B
	ABI 7900 HT Fast	GK003-B
	QuantStudio Fast	GK003-B
	StepOnePlus	GK003-B
	ViiA 7 Fast	GK003-B
Bio-Rad	CFX Connect	GK003-B
	CFX96	GK003-B
	DNA Engine Opticon 2	GK003-B
Stratagene	MX4000	GK003-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK003-C
	Lightcycler 480 (96-well)	GK003-C