



**GeneQuery™ Human Pluripotent Stem Cell qPCR Array Kit  
(GQH-hPSC)  
Catalog #GK002**

**Product Description**

ScienCell's GeneQuery™ Human Pluripotent Stem Cell qPCR array kit (GQH-hPSC) is designed to facilitate gene expression profiling of key genes involved in maintaining pluripotency and self-renewal of hPSC, and genes regulated during differentiation of hPSC. 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<sup>2+</sup>, 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**

<b>Component</b>	<b>Cat #</b>	<b>Quantity</b>	<b>Storage</b>
GeneQuery™ array plate with lyophilized primers	GK002	1	4°C or -20°C
Optical PCR plate seal	N/A	1	RT
Nuclease-free H <sub>2</sub> O	GQ100-1	2	4°C

**Additional Materials Required (Materials Not Included in Kit)**

<b>Component</b>	<b>Recommended</b>
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-hPSC 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

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

**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.)

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

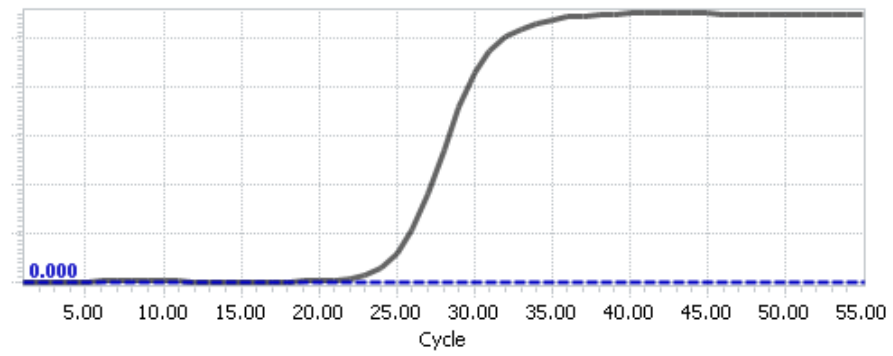
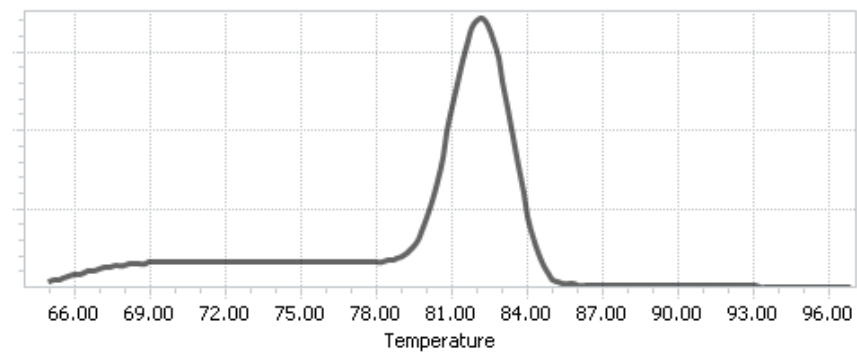


Figure 3. A typical melting peak of a qPCR product.



## **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: ACTB, 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 (ACTB, GAPDH, LDHA, NONO, and PPIH) use the following formula:

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

**Note:**  $\Delta Cq$  (HKG) =  $Cq$  (HKG, experimental sample) -  $Cq$  (HKG, control sample), and  $\Delta Cq$  (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**

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>ACTB</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

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

$$\begin{aligned}\Delta Cq(\text{GOI1}) &= 21.61 - 33.13 \\ &= -11.52\end{aligned}$$

$$\begin{aligned}\Delta Cq(\text{GOI2}) &= 22.19 - 26.47 \\ &= -4.28\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq(\text{GOI1}) &= \Delta Cq(\text{GOI1}) - \Delta Cq(\text{ref}) \\ &= -11.52 - (-0.43) \\ &= -11.09\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq(\text{GOI2}) &= \Delta Cq(\text{GOI2}) - \Delta Cq(\text{ref}) \\ &= -4.28 - (-0.43) \\ &= -3.85\end{aligned}$$

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

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

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

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GeneQuery™ Human Pluripotent Stem Cell 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>	ACTC1	CDX2	EN2	FOXA2	GFAP	ISL1	MIXL1	NOG	PECAM1	SOX1	TBX6	<b><i>ACTB</i></b>
<b>B</b>	AFP	CER1	ENO2	FOXD3	GJA1	KLF4	MYF5	NPM1	POU5F1	SOX2	TCF2	<b><i>GAPDH</i></b>
<b>C</b>	ALB	COL1A1	EOMES	FOXJ3	GSC	LEFTYA	MYOD1	NR5A2	PTF1A	SOX7	TCF3	<b><i>LDHA</i></b>
<b>D</b>	ALDH1A1	COL2A1	ESG1	GATA2	HAND1	LEFTYB	NANOG	OLIG2	REST	SOX17	TDGF1	<b><i>NONO</i></b>
<b>E</b>	BRIX1	CXCR4	ESRRB	GATA4	HDAC2	LIN28	NEUROD	OTX2	REX1	T	TERT	<b><i>PPIH</i></b>
<b>F</b>	CD34	DES	FGF4	GATA6	HLXB9	MEF2C	NCAM1	PAX2	RUNX1	TAT	TUBB3	<b><i>GDC</i></b>
<b>G</b>	CD90	DNMT3B	FGF5	GBX2	HNF4A	MEIS1	NES	PAX6	RUNX2	TBX3	UTF1	<b><i>PPC</i></b>
<b>H</b>	CDH1	EMX2	FLT1	GDF3	IDH1	MESP1	NKX2.5	PDX-1	SALL4	TBX5	VIM	<b><i>NTC</i></b>

\* 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	GK002-A
	ABI 7000	GK002-A
	ABI 7300	GK002-A
	ABI 7500	GK002-A
	ABI 7700	GK002-A
	ABI 7900 HT	GK002-A
	QuantStudio	GK002-A
	ViiA 7	GK002-A
Bio-Rad	Chromo4	GK002-A
	iCycler	GK002-A
	iQ5	GK002-A
	MyiQ	GK002-A
	MyiQ2	GK002-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK002-A
	Matercycler ep realplex 4	GK002-A
Stratagene	MX3000P	GK002-A
	MX3005P	GK002-A

**Plate type B**

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
ABI / Life Tech	ABI 7500 Fast	GK002-B
	ABI 7900 HT Fast	GK002-B
	QuantStudio Fast	GK002-B
	StepOnePlus	GK002-B
	ViiA 7 Fast	GK002-B
Bio-Rad	CFX Connect	GK002-B
	CFX96	GK002-B
	DNA Engine Opticon 2	GK002-B
Stratagene	MX4000	GK002-B

**Plate type C**

<b>Brand</b>	<b>Model</b>	<b>kit catalog #</b>
Roche	Lightcycler 96	GK002-C
	Lightcycler 480 (96-well)	GK002-C