



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 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)
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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

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.

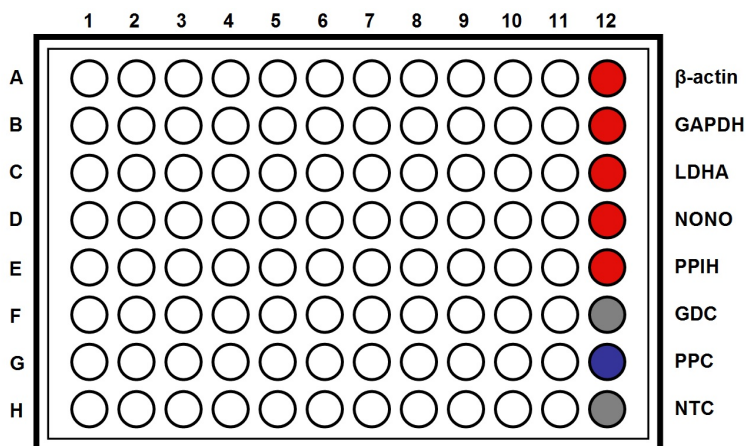


Table 2. Interpretation of control results:

<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.

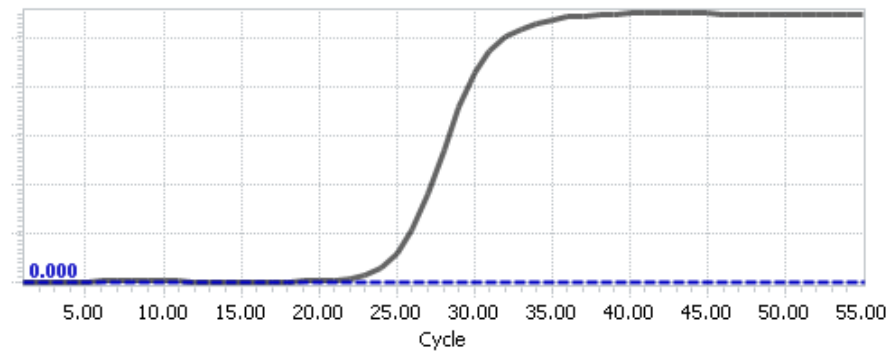
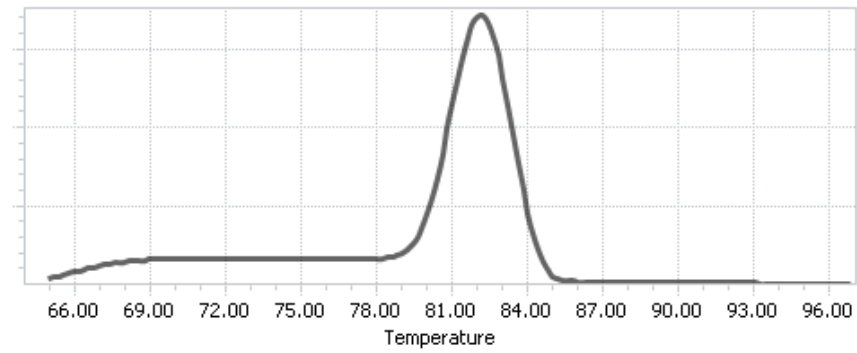


Figure 3. A typical melting peak of a qPCR product.





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

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