GeneQuery™ Human Chondrocyte Biology qPCR Array Kit (GQH-CHN)

Catalog #GK079

Product Description

ScienCell's GeneQuery™ Human Chondrocyte Biology qPCR Array (GQH-CHN) facilitates gene expression profiling of 88 key genes in chondrocyte function. Chondrocytes are embedded in the cartilage matrix and are responsible for producing and maintaining it. Chondrocytes originate in the mesenchyme and are hypertrophic when terminally differentiated during ossification. Brief examples of how included genes may be grouped according to their function in chondrocyte biology are shown below:

- **Chondrogenesis:** COL2A1, ACAN, SOX9, MIA, PTCH1
- **ECM interaction:** RHOA, ITGAV, CD44, PTK2, ITGB1
- **Mechanotransduction:** NFATC4, TRPV4, CREB1, MAPK1, PRKCE
- **Remodeling and repair:** BMP2, HIF1A, MMP9, WNT9A, ICAM1

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^{2+} 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 (β-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 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), 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_2O	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

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.

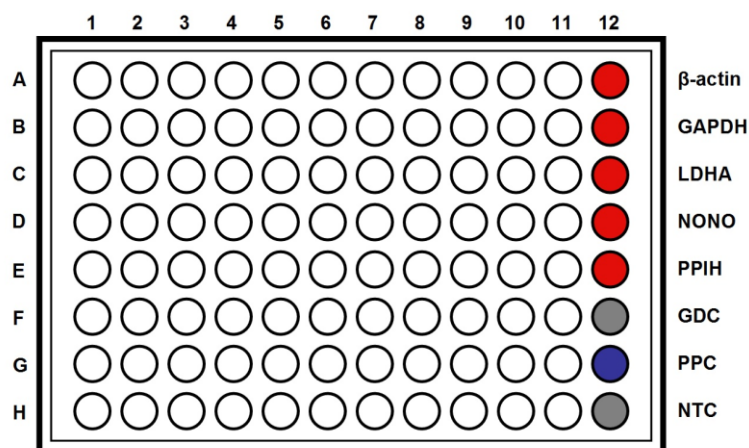Figure 1. Layout of GeneQuery™ qPCR array kit controls.

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.
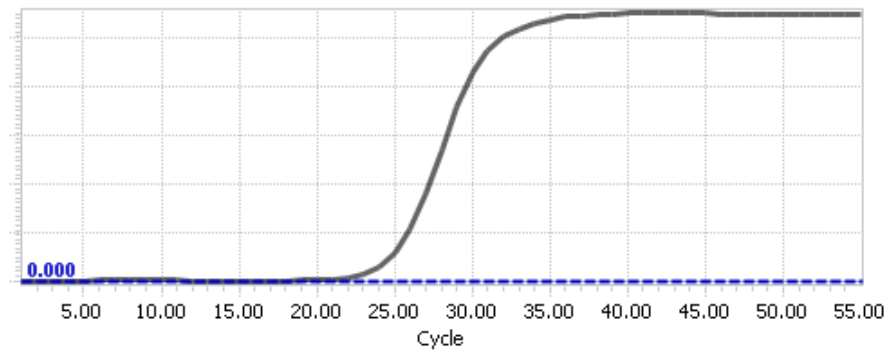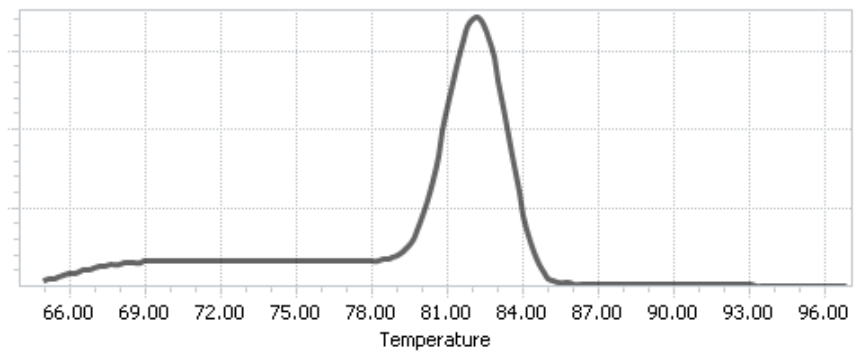

Figure 3. A typical melting peak of a qPCR product.

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, β-actin, 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.

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

   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 ΔCq of the selected housekeeping genes.

   $$\Delta Cq \text{ (ref)} = \text{average } (\Delta Cq \text{ (HKG1)}, \Delta Cq \text{ (HKG2)},......, \Delta Cq \text{ (HKG n)})$$ (n is the number of housekeeping genes selected)

   If using all 5 housekeeping genes included in this kit, β-actin, GAPDH, LDHA, NONO, and PPIH, use the following formula:

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

   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),

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

   $$\Delta\Delta Cq = \Delta Cq \text{ (GOI)} - \Delta Cq \text{ (ref)}$$

   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	*β-actin*	*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

ΔCq (ref) = (ΔCq(β-actin)+ΔCq(GAPDH)+ΔCq(LDHA)+ΔCq(NONO)+ΔCq(PPIH)) /5

$\quad$ = ((17.16-18.20)+(17.84-18.48)+(20.12-20.57)+(19.64-19.50)+(26.40-26.55))/5

$\quad$ = -0.43

ΔCq (GOI1) = 21.61 - 33.13

$\quad$ = -11.52

ΔCq (GOI2) = 22.19 - 26.47

$\quad$ = -4.28

$\Delta\Delta$Cq (GOI1) = ΔCq (GOI1) - ΔCq (ref)

$\quad$ = -11.52 - (-0.43)

$\quad$ = -11.09

$\Delta\Delta$Cq (GOI2) = ΔCq (GOI2) - ΔCq (ref)

$\quad$ = -4.28 - (-0.43)

$\quad$ = -3.85

Normalized GOI1 expression level fold change = $2^{-\Delta\Delta Cq\ (GOI1)}$

$\quad\quad\quad\quad\quad$ = $2^{11.09}$

$\quad\quad\quad\quad\quad$ = 2180

Normalized GOI2 expression level fold change = $2^{-\Delta\Delta Cq\ (GOI2)}$

$\quad\quad\quad\quad\quad$ = $2^{3.85}$

$\quad\quad\quad\quad\quad$ = 14.4

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

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	ACAN	CACNA1C	COL2A1	FGFR3	IGF1R	ITGA6	MAPK3	NFATC4	PTGER4	RUNX2	SOX9	*β-actin*
B	ALPL	CACNA1D	COL4A1	FLNA	IHH	ITGA7	MATN1	NKX3-2	PTH1R	SCNN1A	SP7	*GAPDH*
C	ANXA5	CD151	COL4A2	FLNB	ILK	ITGAV	MATN3	OTOR	PTHLH	SCNN1B	SPARC	*LDHA*
D	ANXA6	CD44	CREB1	FOXC1	ITGA1	ITGB1	MATN4	PRG4	PTK2	SCNN1G	TGFB3	*NONO*
E	AQP1	CD47	CRTAC1	FOXC2	ITGA2	ITGB5	MIA	PRKCA	PXN	SMAD2	TNC	*PPIH*
F	BMP2	CHAD	CTSB	HIF1A	ITGA3	KCNA4	MMP13	PRKCB	RAC1	SMAD3	TRPV4	*GDC*
G	BMP4	CILP	DDR2	IBSP	ITGA4	KCNA6	MMP9	PRKCE	RARG	SOX5	VEGFA	*PPC*
H	CACNA1A	COL11A2	EPYC	ICAM1	ITGA5	MAPK1	NCAM1	PTCH1	RHOA	SOX6	WNT9A	*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	GK079-A
	ABI 7000	GK079-A
	ABI 7300	GK079-A
	ABI 7500	GK079-A
	ABI 7700	GK079-A
	ABI 7900 HT	GK079-A
	QuantStudio	GK079-A
	ViiA 7	GK079-A
Bio-Rad	Chromo4	GK079-A
	iCycler	GK079-A
	iQ5	GK079-A
	MyiQ	GK079-A
	MyiQ2	GK079-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK079-A
	Matercycler ep realplex 4	GK079-A
Stratagene	MX3000P	GK079-A
	MX3005P	GK079-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK079-B
	ABI 7900 HT Fast	GK079-B
	QuantStudio Fast	GK079-B
	StepOnePlus	GK079-B
	ViiA 7 Fast	GK079-B
Bio-Rad	CFX Connect	GK079-B
	CFX96	GK079-B
	DNA Engine Opticon 2	GK079-B
Stratagene	MX4000	GK079-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK079-C
	Lightcycler 480 (96-well)	GK079-C