GeneQuery™ Human Hepatic Steatosis qPCR Array Kit (GQH-HST)

Catalog #GK045

Product Description

ScienCell's GeneQuery™ Human Hepatic Steatosis qPCR Array Kit (GQH-HST) is designed to facilitate gene expression profiling of 88 key genes involved in hepatic steatosis pathogenesis. Hepatic steatosis, also known as fatty liver disease, refers to the accumulation of excessive triglycerides and other lipids in hepatocytes, mainly due to defective fatty acid metabolism. The most common causes of hepatic steatosis include hepatic insulin resistance, alcoholism, obesity or imbalance between energy intake and consumption, and genetic inheritance. Brief examples of how included genes may be grouped are shown below:

- **Insulin signaling pathways:** SOCS3, PPARGC1A, AKT1, MTOR, GSK3B, INSR, IRS1, FOXO1, PIK3R1, SOCS3, SREBF1
- **Cholesterol metabolism:** APOC3, APOA1, APOB, APOE, LDLR, PPARG, SREBF1, SREBF2, NR1H3, CYP2E1
- **Carbohydrate metabolism:** G6PC, G6PD, ACLY, PCK2, PDK4, RBP4, GSK3B
- **Beta-oxidation:** CPT1A, CPT2, FABP1, ACADL, ACOX1, AKT1, CD36, MTOR
- **Adipokine signaling pathways:** PPARGC1A, MTOR, LEPR, IRS1, ADIPOR1, ADIPOR2, NFKB1, SLC2A1, SLC2A4, TNF
- **Hepatotoxicity steatosis:** CD36, FASN, LPL, SCD, PPARA, SREBF1
- **Genes implicated in**
  - **Type II diabetes:** IRS1, INSR, MAPK8, SLC2A2, SLC2A4, XBP1, TNF, PKLR, PNPLA3
  - **Non-alcoholic fatty liver disease:** PNPLA3, SQSTM1, INS, GPT, ADIPOQ, TNF, SLC17A5, LEP, KRT18
  - **Reye's syndrome:** ACADM, OTC, HMGCL, HADHA, OAT, ASS1, HMGCR, CES1
  - **Lipodystrophy:** LMNA, LEP, IRS4, PDE3B, PTRF, GFPT1

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 (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_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 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-HST 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.

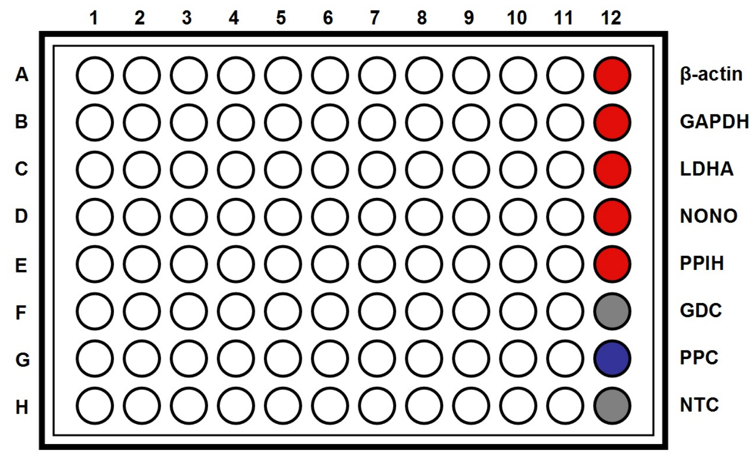


Table 2. Interpretation of control results:

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.

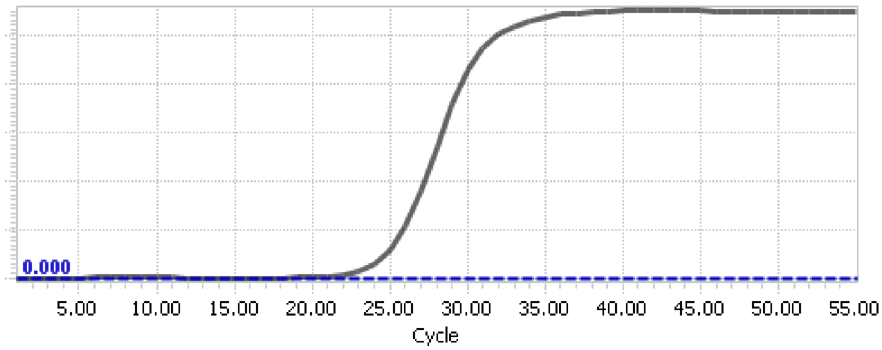
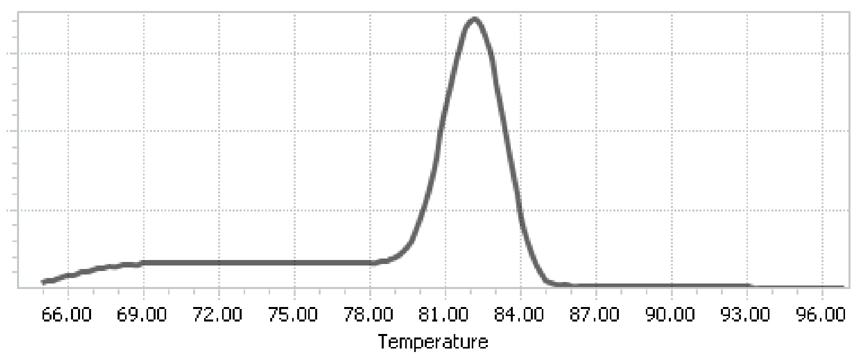


Figure 3. A typical melting peak of a qPCR product.



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, 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.

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

   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, β-actin, GAPDH, LDHA, NONO, and PPIH, use the following formula:

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

   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),

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

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

   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**	***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

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

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

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

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

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

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

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

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

GeneQuery™ Human Hepatic Steatosis qPCR Array Kit (GQH-HST)

Catalog #GK045

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	AKT1	CLEC4M	EIF3E	IFNA2	IRF3	MAPK14	OAS1	PIK3R1	PPP2R1B	RIPK1	STAT6	***β-actin***
B	AKT2	DDX58	GGT1	IFNAR1	JAK1	MAPK3	OAS2	PIK3R2	PPP2R2A	RNASEL	TBK1	***GAPDH***
C	AKT3	EGFR	GPT	IFNAR2	LDLR	MAPK8	OAS3	PIK3R3	PPP2R2B	RXRA	TICAM1	***LDHA***
D	BAD	EIF2AK1	GRB2	IFNB1	MAPK1	MAPK9	OCLN	PIK3R5	PPP2R2C	SCARB1	TLR3	***NONO***
E	CD81	EIF2AK2	HM13	IKBKB	MAPK10	MAVS	PDPK1	PPARA	PPP2R2D	SOS1	TNFRSF1A	***PPIH***
F	CDKN1A	EIF2AK3	IFIT1	IKBKE	MAPK11	NFKB1	PIAS1	PPP2CA	PSME3	SOS2	TRADD	***GDC***
G	CHUK	EIF2AK4	IFIT1B	IKBKG	MAPK12	NFKBIA	PIK3CB	PPP2CB	RAF1	STAT1	TRAF2	***PPC***
H	CLDN1	EIF2S1	IFNA1	IRF1	MAPK13	NR1H3	PIK3CG	PPP2R1A	RELA	STAT2	TYK2	***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	GK045-A
	ABI 7000	GK045-A
	ABI 7300	GK045-A
	ABI 7500	GK045-A
	ABI 7700	GK045-A
	ABI 7900 HT	GK045-A
	QuantStudio	GK045-A
	ViiA 7	GK045-A
Bio-Rad	Chromo4	GK045-A
	iCycler	GK045-A
	iQ5	GK045-A
	MyiQ	GK045-A
	MyiQ2	GK045-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK045-A
	Matercycler ep realplex 4	GK045-A
Stratagene	MX3000P	GK045-A
	MX3005P	GK045-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK045-B
	ABI 7900 HT Fast	GK045-B
	QuantStudio Fast	GK045-B
	StepOnePlus	GK045-B
	ViiA 7 Fast	GK045-B
Bio-Rad	CFX Connect	GK045-B
	CFX96	GK045-B
	DNA Engine Opticon 2	GK045-B
Stratagene	MX4000	GK045-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK045-C
	Lightcycler 480 (96-well)	GK045-C