Rev.0

GeneQuery™ Human FAS/FASL Signaling qPCR Array Kit (GQH-FAS)
Catalog #GK149

Product Description

ScienCell's GeneQuery™ Human FAS/FASL Signaling qPCR Array Kit (GQH-FAS) profiles 88 key genes involved in the human FAS/FASL signaling pathway. The Fas receptor (CD95, tumor necrosis factor receptor superfamily member 6) is a death receptor located on the surface of various cells. It initiates a signal transduction pathway through its interaction with its ligand, FasL (FasL/CD95L). The Fas/FasL signaling pathway is a major regulator of apoptosis and plays a significant role in various cellular processes, including differentiation and survival. While Fas is primarily known for inducing cell death, it also transmits proliferative and activating signals in normal human cells. The downstream signaling of Fas is complex and regulated by multiple pathway components. GQH-FAS provides a comprehensive tool for examining the expression profiles of key genes involved in the Fas/FasL signaling pathway, facilitating the study of this pathway regulation. Brief examples of how genes may be grouped according to their functions are shown below:

- **Receptors:** TNFRSF1B, FAS
- **Receptor Transducers**: FADD, MYD88, RIPK1, TRAF1, TRAF2, IRAK1
- **Ligands:** FASLG, TNFSF10, TNF
- **Transcription Factors:** ETS1, JUN, ATF2, RELA, RELB

***Note*:** all gene names follow their official symbols by the Human Genome Organization Gene Nomenclature Committee (HGNC).

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- 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.

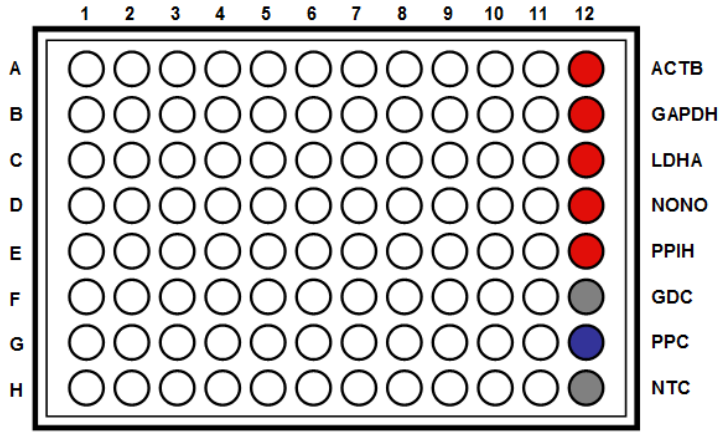


Table 2. Interpretation of control results:

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.

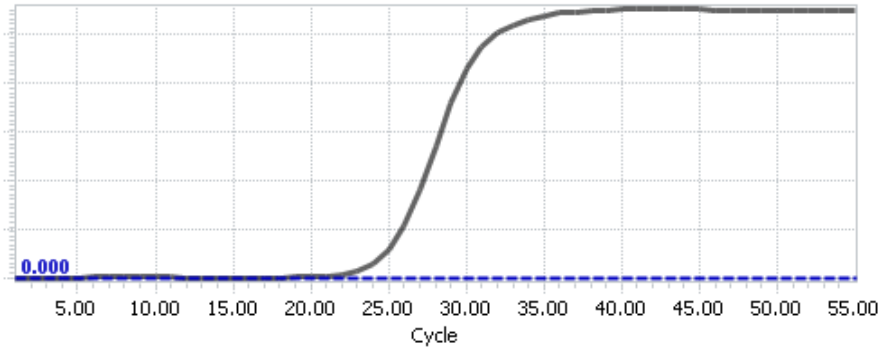
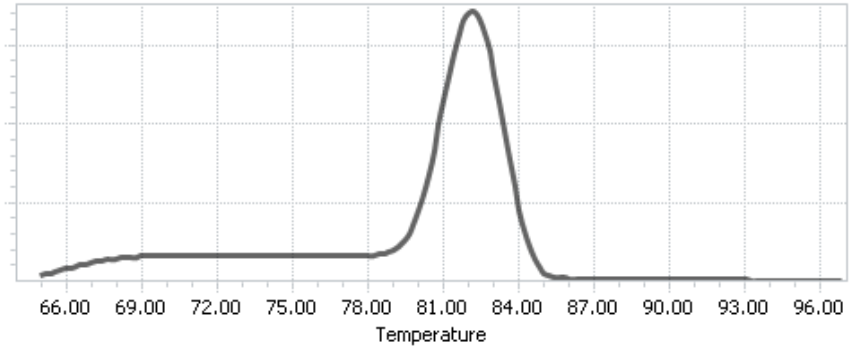


Figure 3. A typical melting peak of a qPCR product.



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.

   ΔCq (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.

   ΔCq (ref) = average (ΔCq (HKG1), ΔCq (HKG2),....., ΔCq (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:

   ΔCq (ref) = (ΔCq(ACTB)+ΔCq(GAPDH)+ΔCq(LDHA)+ΔCq(NONO)+ΔCq(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),

   ΔCq (GOI) = Cq (GOI, experimental sample) - Cq (GOI, control sample)

   ΔΔCq = ΔCq (GOI) - ΔCq (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

ΔCq (ref) = (ΔCq(ACTB)+ΔCq(GAPDH)+ΔCq(LDHA)+ΔCq(NONO)+ΔCq(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

ΔCq (GOI1) = 21.61 - 33.13
= -11.52

ΔCq (GOI2) = 22.19 - 26.47
= -4.28

ΔΔCq (GOI1) = ΔCq (GOI1) - ΔCq (ref)
= -11.52 - (-0.43)
= -11.09

ΔΔCq (GOI2) = ΔCq (GOI2) - ΔCq (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.

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

***Note*:** all gene names follow their official symbols by HGNC

	1	2	3	4	5	6	7	8	9	10	11	12
A	ADAM10	BCL2L1	CASP2	CHUK	EGR2	IGF1	LMNA	MAPK3	NGF	RELA	TNFSF10	***ACTB***
B	AKT1	BID	CASP3	CRADD	EGR3	IKBKB	LMNB1	MAPK8	PAK1	RELB	TP53	***GAPDH***
C	ANGPT2	BIRC2	CASP6	CYCS	ETS1	IKBKG	MAP2K1	MAX	PAK2	RIPK1	TRADD	***LDHA***
D	APAF1	BIRC3	CASP7	DAXX	FADD	IRAK2	MAP2K4	MCL1	PARP1	RIPK2	TRAF1	***NONO***
E	ATF2	BIRC5	CASP8	DFFA	FAF2	IRAK3	MAP3K1	MYC	PRKDC	SP1	TRAF2	***PPIH***
F	BAD	BTK	CASP9	DFFB	FAS	IRF1	MAP3K14	MYD88	RAF1	SUMO1	TRAF3	***GDC***
G	BAX	CASP1	CAPN1	DIABLO	FASLG	IRF2	MAP3K5	NFKB1	RB1	TNF	UBE2I	***PPC***
H	BCL2	CASP10	CFLAR	EGR1	HRK	JUN	MAP3K7	NFKB2	REL	TNFRSF1B	XIAP	***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	GK101-A
	ABI 7000	GK101-A
	ABI 7300	GK101-A
	ABI 7500	GK101-A
	ABI 7700	GK101-A
	ABI 7900 HT	GK101-A
	QuantStudio	GK101-A
	ViiA 7	GK101-A
Bio-Rad	Chromo4	GK101-A
	iCycler	GK101-A
	iQ5	GK101-A
	MyiQ	GK101-A
	MyiQ2	GK101-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK101-A
	Matercycler ep realplex 4	GK101-A
Stratagene	MX3000P	GK101-A
	MX3005P	GK101-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK101-B
	ABI 7900 HT Fast	GK101-B
	QuantStudio Fast	GK101-B
	StepOnePlus	GK101-B
	ViiA 7 Fast	GK101-B
Bio-Rad	CFX Connect	GK101-B
	CFX96	GK101-B
	DNA Engine Opticon 2	GK101-B
Stratagene	MX4000	GK101-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK101-C
	Lightcycler 480 (96-well)	GK101-C