GeneQuery™ Human SARS-CoV-2 Interacting Genes qPCR Array Kit (GQH-SCV)

Catalog #GK132

Product Description

Coronaviruses are a family of large RNA viruses with sizes ranging from 26 to 32 kb. These viruses are zoonotic and in humans can cause respiratory infections. In December 2019, a new deadly coronavirus known as SARS-CoV-2, which has a high sequence similarity to SARS-CoV, was identified. SARS-CoV-2 has caused a pneumonia outbreak known as Covid-19 in Wuhan, China and spread globally. Currently, the limited understanding of the genetic basis of the virus-host cells interaction and the host's response has become a major challenge in SARS-CoV-2 research and therapeutic treatments.

ScienCell's GeneQuery™ Human SARS-CoV-2 Interacting Genes qPCR Array Kit (GQH-SCV) is designed to facilitate gene expression profiling of 86 genes that were identified as SARS-CoV-2 interacting genes[1-3]. Additionally, two SARS-CoV-2 genes, the nucleocapsid (N) and the spike (S) genes, were included in the panel allowing quantification of the virus presence in the samples. Brief examples of how included genes may be grouped according to their functions are shown below:

- **SAR-CoV2 virus genes:** SARS-CoV-2 N gene, SARS-CoV-2 S gene
- **Viral entry-associated proteins:** ACE2, AGTR2, TMPRSS2, CTSL, CTSB
- **Endocytosis:** ACSL5, CRIP2, HSD17B4, EPHX1, MCCC2, GSTA4, ACACA, HGD
- **Pulmonary surfactants:** SFTPA1, SFTPB, SFTPC
- **Pro-coagulation and profibrotic genes:** PLSCR1, SERPINE1, THBS1, FGB, PLAT, PLAU, TGFB1, CTGF, PDGFA, COL10A1, COL11A1
- **Innate immune signaling pathway:** DDX58, IRF7, STAT1, G1P2, GBP1, IFNG, MX1, ISG20, OAS1, IDO1, IRAK3, NOS2, TNFSF10, RNF128, MCL1
- **Inflammatory cytokines/chemokines and regulation:** IL1A, IL1B, CXCL10, IL6, CXCL8, IL10, IL12A, CCL2, IFNG, IFIT1, TNF, G1P2, GBP1
- **Cell cycle and development:** AREG, CDK1, CDKN3, CKS2, CCNE1
- **Epigenetic and gene expression regulators:** CEP250, CEP350, CNTRL, NINL, PCNT, CDK5RAP2, CENPF, BRD2, HDAC2
- **Protein translational machinery:** EEF1A1, EIF4B, MIF4GD, DDX5
- **Vesicle trafficking and protein degradation:** GOLGA2, GOLGB1, GORASP1, GCC2, VPS39, SLC27A2, SLC25A21, ACADM, DCTN2
- **Mitochondria functions:** NDUFB9, NDUFAF2, NDUFA10, ABCC1, ALG11, MRPS27, MRPS5

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.

Reference

1. D. E. Gordon *et al.* A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. ***Nature*** (2020). https://doi.org/10.1038/s41586-020-2286-9
2. P. H. Guzzi *et al.* Master Regulator Analysis of the SARS-CoV-2/Human Interactome. *J. Clin. Med.* **2020**, *9*, 982.
3. A. Lang et al. Functional Genomics Highlights Differential Induction of Antiviral Pathways in the Lungs of SARS-CoV-Infected Macaques. *Plos Pathogens.* **2007**, *8*, 1129-41.

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	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

For Research Use Only. Not for use in 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.

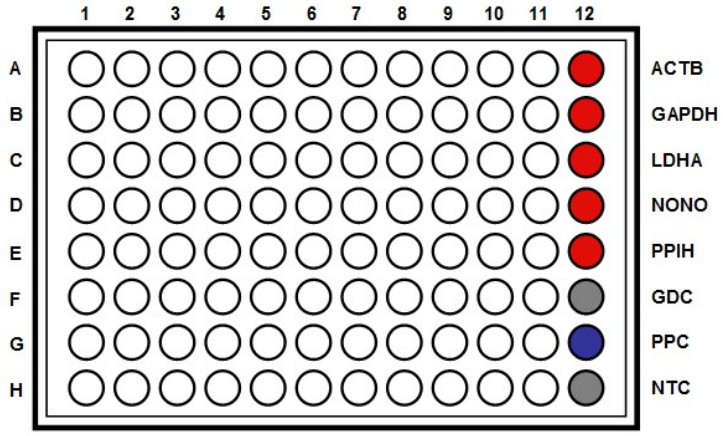


Table 2. Interpretation of control results:

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.

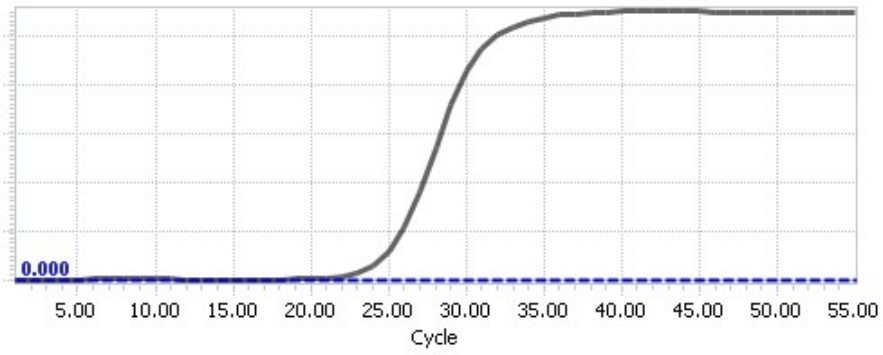
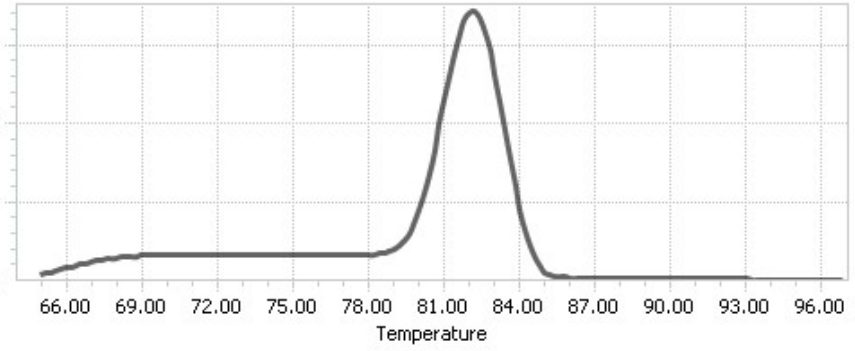


Figure 3. A typical melting peak of a qPCR product.



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.

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

   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, ACTB, GAPDH, LDHA, NONO, and PPIH, use the following formula:

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

   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**	***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

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

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

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

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

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

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 SARS-CoV-2 Interacting Genes qPCR Array Plate Layout*
(***8 controls*** in Bold and Italic)

	1	2	3	4	5	6	7	8	9	10	11	12
A	N gene	ALG11	CENPF	CTGF	EEF1A1	GOLGB1	IFNG	ISG20	NDUFAF2	PLAU	SLC27A2	***ACTB***
B	S gene	AREG	CEP250	CTSB	EIF4B	GORASP1	IL10	MCCC2	NDUFB9	PLSCR1	STAT1	***GAPDH***
C	ABCC1	BRD2	CEP350	CTSL	EPHX1	GSTA4	IL12A	MCL1	NINL	RNF128	TGFB1	***LDHA***
D	ACACA	CCL2	CKS2	CXCL10	FGB	HDAC2	IL1A	MIF4GD	NOS2	SERPINE1	THBS1	***NONO***
E	ACADM	CCNE1	CNTRL	CXCL8	G1P2	HGD	IL1B	MRPS27	OAS1	SFTPA1	TMPRSS2	***PPIH***
F	ACE2	CDK1	COL10A1	DCTN2	GBP1	HSD17B4	IL6	MRPS5	PCNT	SFTPB	TNF	***GDC***
G	ACSL5	CDK5RAP2	COL11A1	DDX5	GCC2	IDO1	IRAK3	MX1	PDGFA	SFTPC	TNFSF10	***PPC***
H	AGTR2	CDKN3	CRIP2	DDX58	GOLGA2	IFIT1	IRF7	NDUFA10	PLAT	SLC25A21	VPS39	***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	GK132-A
	ABI 7000	GK132-A
	ABI 7300	GK132-A
	ABI 7500	GK132-A
	ABI 7700	GK132-A
	ABI 7900 HT	GK132-A
	QuantStudio	GK132-A
	ViiA 7	GK132-A
Bio-Rad	Chromo4	GK132-A
	iCycler	GK132-A
	iQ5	GK132-A
	MyiQ	GK132-A
	MyiQ2	GK132-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK132-A
	Matercycler ep realplex 4	GK132-A
Stratagene	MX3000P	GK132-A
	MX3005P	GK132-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK132-B
	ABI 7900 HT Fast	GK132-B
	QuantStudio Fast	GK132-B
	StepOnePlus	GK132-B
	ViiA 7 Fast	GK132-B
Bio-Rad	CFX Connect	GK132-B
	CFX96	GK132-B
	DNA Engine Opticon 2	GK132-B
Stratagene	MX4000	GK132-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK132-C
	Lightcycler 480 (96-well)	GK132-C