



GeneQuery™ Human Cellular Senescence qPCR Array Kit (GQH-CS)

Catalog #GK151

Product Description

ScienCell's GeneQuery™ Human Cellular Senescence qPCR Array Kit (GQH-CS) profiles 88 genes involved in human cellular senescence. Cellular senescence is defined as a stable, stress-induced cell cycle arrest that functions to eliminate the proliferation of damaged cells. Common triggers include DNA damage, telomere erosion, oncogene activation, and oxidative stress. Senescent cells display distinctive features, including enlarged morphology, chromatin alterations, and secretion of bioactive molecules via the senescence-associated secretory phenotype (SASP), which influences tissue microenvironment and aging-related diseases. GQH-CS provides a powerful and comprehensive tool for simultaneously quantifying the major drivers, effectors, and markers of senescence across multiple pathways. This kit enables researchers to accurately profile senescence-associated gene expression changes in models of replicative senescence, oncogene-induced senescence (OIS), therapy-induced senescence (TIS), and age-related tissue dysfunction. Brief functional groupings are shown below:

- **Core cell-cycle arrest:** CDKN1A, CDKN2A, CDKN2B, RB1, RBL1, RBL2
- **DNA Damage & p53 Pathway:** TP53, ATM, CHEK2, MDM2
- **Classic SASP / Inflammation:** IL6, IL8, SERPINE1, TGFB1, IGFBP7
- **Oxidative Stress & Metabolic Regulators:** SIRT1, SOD1, SOD2, NOX4, GLB1
- **Oncogene & Growth Signaling Pathways:** HRAS, MYC, AKT1, PIK3CA, PTEN, MDM2

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 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 (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 predisposed 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	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-SC 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 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.

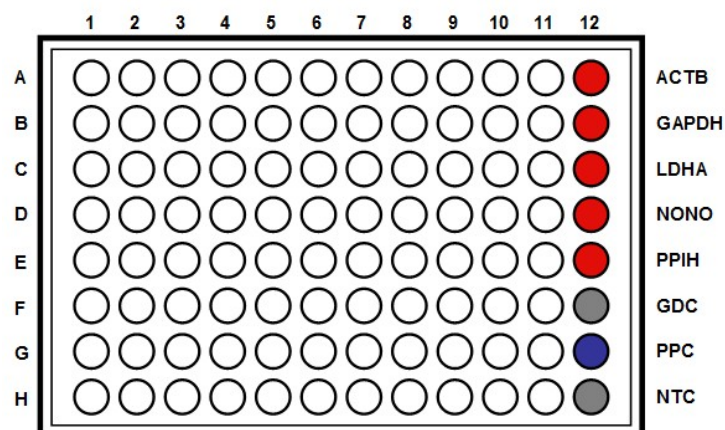


Table 2. Interpretation of control results:

<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.

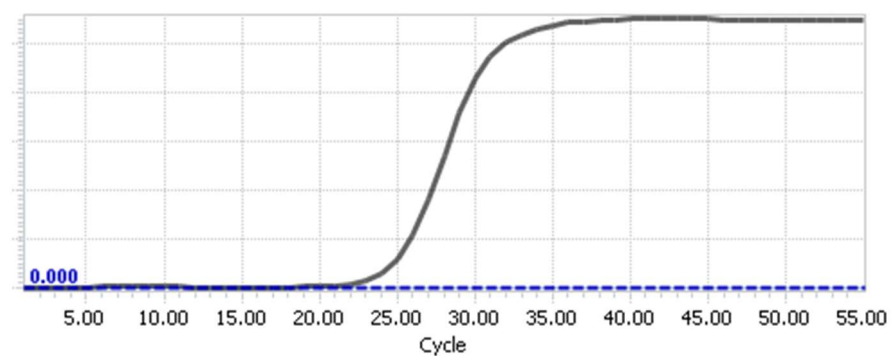
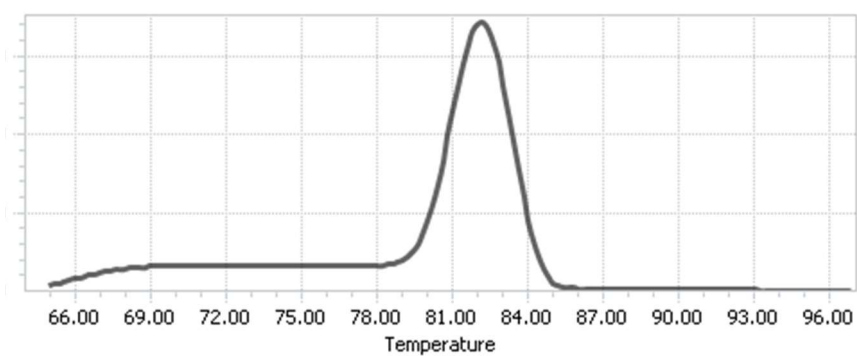


Figure 3. A typical melting peak of a qPCR product.



Quantification Method: Comparative $\Delta\Delta Cq$ (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.

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

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.

$\Delta Cq \text{ (ref)} = \text{average } (\Delta Cq \text{ (HKG1)}, \Delta Cq \text{ (HKG2)}, \dots, \Delta Cq \text{ (HKG } n))$ (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:

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

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),

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

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

$$\text{Normalized GOI expression level fold change} = 2^{-\Delta\Delta Cq}$$

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 $\Delta\Delta Cq$ (Quantification Cycle Value) MethodTable 3. Cq (Quantification Cycle) values of 2 genes-of-interest and 5 housekeeping genes obtained for experimental and control samples.

Samples	Genes of Interest		Housekeeping Genes				
	GOI1	GOI2	<i>ACTB</i>	<i>GAPDH</i>	<i>LDHA</i>	<i>NONO</i>	<i>PPIH</i>
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

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

$$\begin{aligned}\Delta Cq(\text{GOI1}) &= 21.61 - 33.13 \\ &= -11.52\end{aligned}$$

$$\begin{aligned}\Delta Cq(\text{GOI2}) &= 22.19 - 26.47 \\ &= -4.28\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq(\text{GOI1}) &= \Delta Cq(\text{GOI1}) - \Delta Cq(\text{ref}) \\ &= -11.52 - (-0.43) \\ &= -11.09\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq(\text{GOI2}) &= \Delta Cq(\text{GOI2}) - \Delta Cq(\text{ref}) \\ &= -4.28 - (-0.43) \\ &= -3.85\end{aligned}$$

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

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

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 Cellular Senescence 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	ABL1	CCND1	CDKN1B	COL1A1	FN1	IGF1	IRF3	MDM2	PLAU	SIRT1	TGFB1	<i>ACTB</i>
B	AKT1	CCNE1	CDKN1C	COL3A1	FOXO4	IGF1R	IRF5	MORC3	PRKCD	SMAD3	TGFB1I1	<i>GAPDH</i>
C	ALDH1A3	CD44	CDKN2A	CREG1	GADD45A	IGFBP3	IRF7	MYC	PTEN	SOD1	THBS1	<i>LDHA</i>
D	ATM	CDC25C	CDKN2B	E2F1	GLB1	IGFBP5	LMNB1	NBN	RB1	SOD2	TP53	<i>NONO</i>
E	BMI1	CDK2	CDKN2C	E2F3	GSK3B	IGFBP7	MAP2K1	NFKB1	RBL1	SPARC	TP53BP1	<i>PPIH</i>
F	CALR	CDK4	CDKN2D	EGR1	HRAS	IL6	MAP2K3	NOX4	RBL2	TBX2	TWIST1	<i>GDC</i>
G	CCNA2	CDK6	CHEK1	ETS1	ID1	IL8	MAP2K6	PCNA	SERPINE2	TBX3	VIM	<i>PPC</i>
H	CCNB1	CDKN1A	CHEK2	ETS2	IFNG	ING1	MAPK14	PIK3CA	SERPINE1	TERT	WRN	<i>NTC</i>

* 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