GeneQuery™ Human Cytoskeletal Remodeling qPCR Array Kit (GQH-CYT)

Catalog #GK104

Product Description

ScienCell's GeneQuery™ Human Cytoskeletal Remodeling qPCR Array Kit (GQH-CYT) profiles 88 key genes involved in cytoskeletal remodeling with a particular focus on Rho GTPase actin cytoskeleton remodeling. The cytoskeleton is responsible for maintaining cell shape, scaffolding, and regulating intracellular transport. It is also involved in numerous signaling pathways, cellular division, vesicular trafficking, and motility. Microfilaments, which are primarily actin polymers, are part of the cytoskeleton and regulated by the Rho family of GTPases. Brief examples of how included genes may be categorized are shown below:

- **Cytoskeletal components:** ACTA1, ACTB, ACTG1, ACTN1, MYH10, MYL1
- **Integrins:** ITGA1, ITGA3, ITGAV, ITGB1, ITGB3
- **Extracellular matrix components:** COL1A1, COL2A1, COL4A1, LAMA1, LAMB1
- **Rho signaling molecules:** CAV1, CDC42, RAC1, RHOA, ROCK1, ROCK2
- **Transcription factors:** CDX1, CDX2, SP1, TCF7, TCF7L1

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.

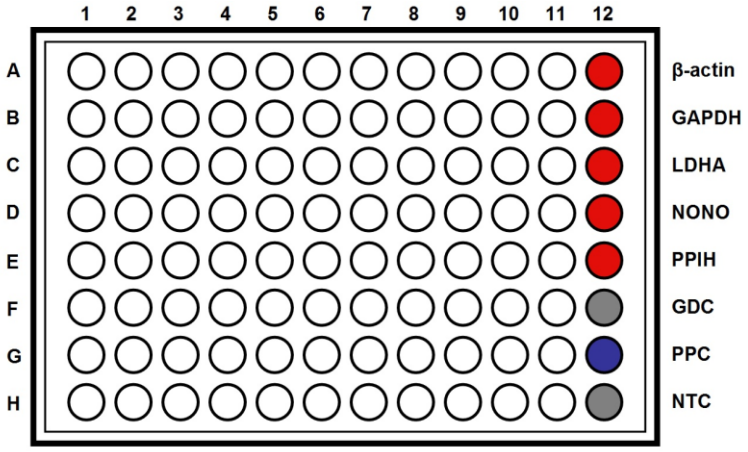


Table 2. Interpretation of control results:

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.

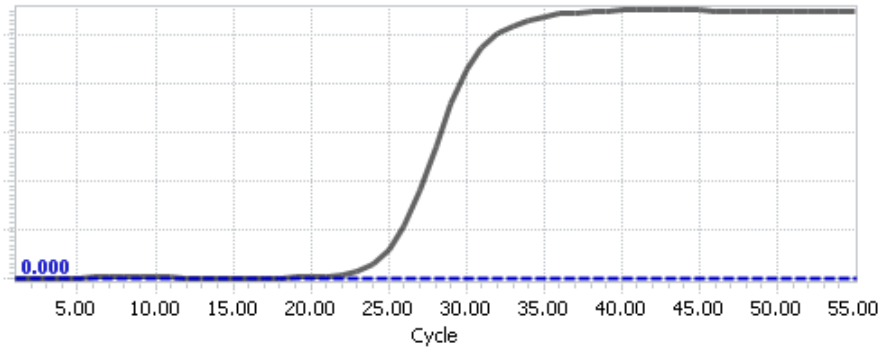
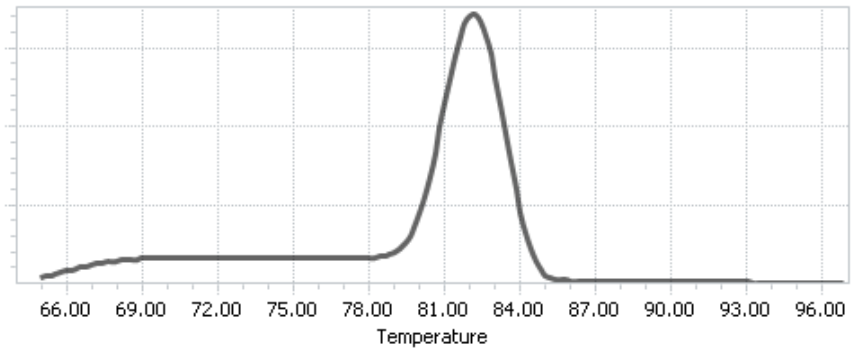


Figure 3. A typical melting peak of a qPCR product.



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.

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

   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

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

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

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

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

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

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 Cytoskeletal Remodeling qPCR Array Plate Layout* (***8 controls*** in Bold and Italic)

	1	2	3	4	5	6	7	8	9	10	11	12
A	ACTA1	ACTN3	CFL1	COL4A1	ITGA3	LAMB1	MYH2	MYL12B	MYLK	PTK2	SRC	***β-actin***
B	ACTA2	ACTN4	CFL2	COL4A2	ITGA5	LAMC1	MYH3	MYL2	MYLK2	PXN	TCF7	***GAPDH***
C	ACTB	ACTR3B	CLDN4	COL4A5	ITGA7	LEF1	MYH6	MYL3	MYLK3	RAC1	TCF7L1	***LDHA***
D	ACTC1	ARHGDIA	CLDN5	FLNA	ITGA8	LIMK1	MYH7	MYL4	MYLPF	RHOA	TCF7L2	***NONO***
E	ACTG1	CAV1	CLDN7	FN1	ITGAV	MAPK1	MYH7B	MYL5	NECTIN1	ROCK1	TJP1	***PPIH***
F	ACTG2	CDC42	COL1A1	GRB2	ITGB1	MAPK3	MYH8	MYL6	NKX2-1	ROCK2	TLN1	***GDC***
G	ACTN1	CDX1	COL1A2	ITGA1	ITGB3	MYH10	MYH9	MYL6B	PAK1	SNAI1	TLN2	***PPC***
H	ACTN2	CDX2	COL2A1	ITGA2	LAMA1	MYH11	MYL1	MYL9	PIP5K1C	SP1	VCL	***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	GK104-A
	ABI 7000	GK104-A
	ABI 7300	GK104-A
	ABI 7500	GK104-A
	ABI 7700	GK104-A
	ABI 7900 HT	GK104-A
	QuantStudio	GK104-A
	ViiA 7	GK104-A
Bio-Rad	Chromo4	GK104-A
	iCycler	GK104-A
	iQ5	GK104-A
	MyiQ	GK104-A
	MyiQ2	GK104-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK104-A
	Matercycler ep realplex 4	GK104-A
Stratagene	MX3000P	GK104-A
	MX3005P	GK104-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK104-B
	ABI 7900 HT Fast	GK104-B
	QuantStudio Fast	GK104-B
	StepOnePlus	GK104-B
	ViiA 7 Fast	GK104-B
Bio-Rad	CFX Connect	GK104-B
	CFX96	GK104-B
	DNA Engine Opticon 2	GK104-B
Stratagene	MX4000	GK104-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK104-C
	Lightcycler 480 (96-well)	GK104-C