Rev. 1

GeneQuery™ Human Urothelial Cell Biology qPCR Array Kit (GQH-URO)

Catalog #GK082

Product Description

ScienCell's GeneQuery™ Human Urothelial Cell Biology qPCR Array Kit (GQH-URO) is designed to facilitate gene expression profiling of key genes involved in both normal and abnormal urothelial cell function. Urothelial cells are the cells lining the surface of the urinary bladder. They are the first line of bladder defense and serve as an interface between pathogens. They are equipped with several defense mechanisms to prevent adherence of pathogens and maintain impermeability to urinary solutes. Brief examples of how included genes may be grouped according to their functions are shown below:

- **Urothelial cell development and regulation:** EGFR, FGF10, HBEGF, IL22, ISL1, MAPK1, NGFR, RBL1, SHH, SIX1, SOX9, TBX18, TNXB
- **Prominently expressed in normal urothelium/urogenital tract:** CD44, ESR2, RBL1, TRPV1
- **Involved in bladder distention/function:** BDKRB2, P2RX3, TRPV4
- **Cytokeratins:** KRT13, KRT17, KRT18, KRT19, KRT20, KRT5, KRT7, KRT8
- **Uroplakins:** UPK1A, UPK1B, UPK2, UPK3A, UPK3B
- **Syndromes/abnormalities/infections:** ACTA2, ACTG2, CHRM3, CXCL8, HNF1B, HPSE2, LRIG2, TP63, TRPA1, VEGFA
- **Misregulation found in carcinomas:** ALKBH3, ATF2, AURKA, BMP10, CD44, CDC6, CDKN1B, CDKN2A, EIF3D, ESR1, FGFR3, GJB2, HEPACAM, HGF, HRAS, KRAS, LIN28B, LYPD3, MACROH2A1, MAPK1, MMP2, MMP9, MSI1, NRAS, PIK3CA, PTEN, RECK, ROCK2, RRM2, TSC1
- **Cancer Associated Cell Surface Proteins:** CD37, CD53, CD63, CD9
- **Cancer Prognostic Biomarker:** ALDH1A1, ALDH1A3, BSG, HOXA9, MKI67, MSI2, SLIT3, TIMP1, TP53, TRPM8, TRPV2

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 (ACTB, 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	Cat #	Quantity	Storage
GeneQuery™ array plate with lyophilized primers	GK082	1	4°C or -20°C
Optical PCR plate seal	N/A	1	RT
Nuclease-free H_2O	GQ100-1	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 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-URO 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.

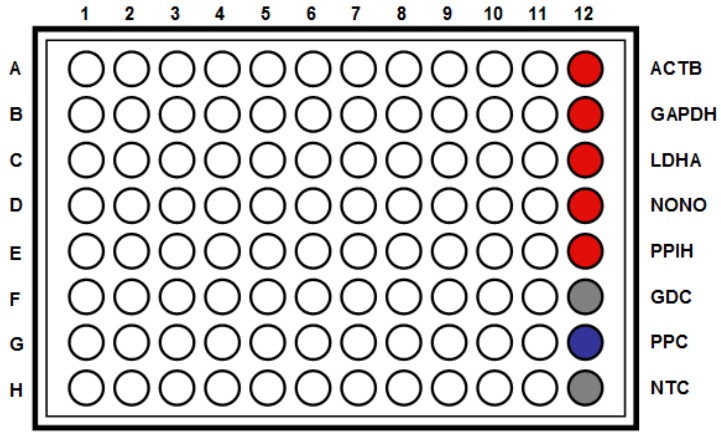


Table 2. Interpretation of control results:

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.

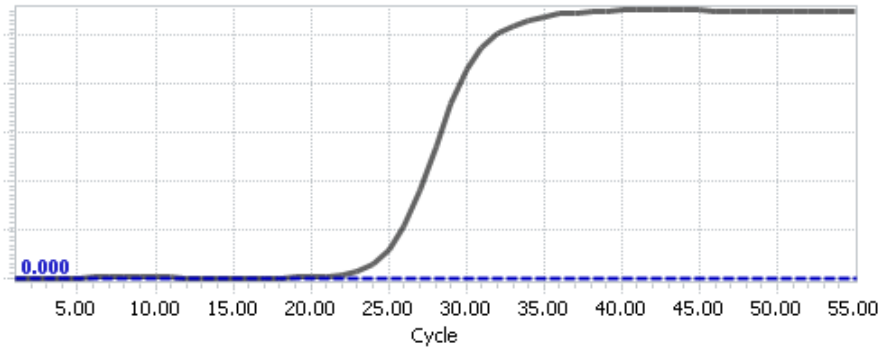
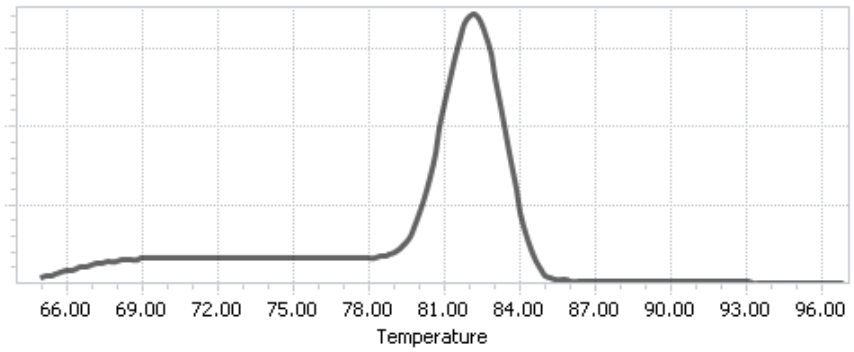


Figure 3. A typical melting peak of a qPCR product.



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: 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 Osteogenic Differentiation qPCR Array Plate Layout* (***8 controls*** in Bold and Italic)

	1	2	3	4	5	6	7	8	9	10	11	12
A	ACTA2	BMP10	CDC6	EIF3D	HGF	KRT13	LIN28B	MSI1	RECK	TIMP1	TRPV4	***ACTB***
B	ACTG2	BNC2	CDKN1B	ESR1	HNF1B	KRT17	LRIG2	MSI2	ROCK2	TNXB	TSC1	***GAPDH***
C	ALDH1A1	BSG	CDKN2A	ESR2	HOXA9	KRT18	LYPD3	NGFR	RRM2	TP53	UPK1A	***LDHA***
D	ALDH1A3	CD37	CHRM3	FGF10	HPSE2	KRT19	MACROH2A1	NRAS	SHH	TP63	UPK1B	***NONO***
E	ALKBH3	CD44	CIP2A	FGFR3	HRAS	KRT20	MAPK1	P2RX3	SIX1	TRPA1	UPK2	***PPIH***
F	ATF2	CD53	CSTB	GJB2	IL22	KRT5	MKI67	PIK3CA	SLIT3	TRPM8	UPK3A	***GDC***
G	AURKA	CD63	CXCL8	HBEGF	ISL1	KRT7	MMP2	PTEN	SOX9	TRPV1	UPK3B	***PPC***
H	BDKRB2	CD9	EGFR	HEPACAM	KRAS	KRT8	MMP9	RBL1	TBX18	TRPV2	VEGFA	***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	GK082-A
	ABI 7000	GK082-A
	ABI 7300	GK082-A
	ABI 7500	GK082-A
	ABI 7700	GK082-A
	ABI 7900 HT	GK082-A
	QuantStudio	GK082-A
	ViiA 7	GK082-A
Bio-Rad	Chromo4	GK082-A
	iCycler	GK082-A
	iQ5	GK082-A
	MyiQ	GK082-A
	MyiQ2	GK082-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK082-A
	Matercycler ep realplex 4	GK082-A
Stratagene	MX3000P	GK082-A
	MX3005P	GK082-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK082-B
	ABI 7900 HT Fast	GK082-B
	QuantStudio Fast	GK082-B
	StepOnePlus	GK082-B
	ViiA 7 Fast	GK082-B
Bio-Rad	CFX Connect	GK082-B
	CFX96	GK082-B
	DNA Engine Opticon 2	GK082-B
Stratagene	MX4000	GK082-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK082-C
	Lightcycler 480 (96-well)	GK082-C