Rev. 1

GeneQuery™ Human PI3K/AKT Signaling Pathway qPCR Array Kit (GQH-AKT)

Catalog #GK140

Product Description

ScienCell's GeneQuery™ Human PI3K/AKT Signaling Pathway qPCR Array Kit (GQH-AKT) profiles 88 key genes associated with the PI3K (Phosphoinositide 3-kinases)/Akt signaling pathway, which is an intracellular signal transduction pathway that promotes metabolism, proliferation, cell survival, growth and angiogenesis. In the pathway, PI3K is activated by many types of extracellular signals or toxic insults. Activated PI3K catalyzes the production of phosphatidylinositol-3,4,5-triphosphate (PIP3) at the cell membrane. PIP3 in turn serves as a second messenger that helps to activate Akt. Once active, Akt can control key cellular processes by phosphorylating substrates involved in apoptosis, protein synthesis, metabolism, and cell cycle. Brief examples of how included genes may be grouped according to their functions are shown below:

- **PI3K and AKT Family Genes and Regulators:** AKT1, AKT2, AKT3, BTK, GRB2, HSPB1, ILK, PDPK1, PIK3CA, PIK3CG, PIK3R1, PIK3R2, PAK1, PRKCA, PRKCB, PRKCZ, PTEN
- **IGF/AKT Signaling:** CSNK2A1, ELK1, FOS, GRB2, HRAS, IGF1, IGF1R, IRS1, JUN, MAP2K1, MAPK3, MAPK8, PTPN11, RAF1, RASA1, SHC1, SOS1, SRF
- **AKT/mTOR Signaling:** EIF4B, EIF4E, MTOR, PTEN, RHEB, RPS6KB1, TSC1, TSC2
- **GSK3 Signaling Pathway:** ADAR, APC, CCND1, CD14, CTNNB1, EIF2AK2, GJA1, GSK3B, IRAK1, MYD88, NFKB1, TIRAP, TLR4
- **Cell Cycle Arrest and Apoptosis:** CDKN1B, FASLG, FOXO3, GRB2, ILK, ITGB1, MAPK1, MAPK3, PTEN, PTK2, RBL2, SHC1, SOS1
- **BAD Phosphorylation and Cell Survival:** BAD, GRB2, HRAS, IGF1R, IRS1, MAP2K1, MAPK1, MAPK3, RAF1, RPS6KA1, SHC1, SOS1, YWHAH
- **Cell Migration:** CDC42, PDGFRA, RAC1, RHOA, WASL
- **Other Downstream Signaling Pathways:** EIF4E, MTOR, IRS1, MAPK1, MAPK14, MAPK3, PRKCA, PTEN, RPS6KB1
- **Other Genes Involved in the AKT Signaling Pathway:** CASP9, CHUK, FOXO1, NFKBIA, DPYSL2, GSK3A, FOXO4, MDM2, NOS1, NOS3, FOXA2, NR4A1, INPP4B, RPS6KB2, HTT, FKBP5, CREB1, IKBKB

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 porcine 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	Cat #	Quantity	Storage
GeneQuery™ array plate with lyophilized primers	GK140	1	4°C or -20°C
Optical PCR plate seal	N/A	1	RT
Nuclease-free H_2O	GQ100-1	2	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-AKT 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 product should be stored at 4°C and is good for up to 12 months. For long-term storage (>1 year), store the product 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

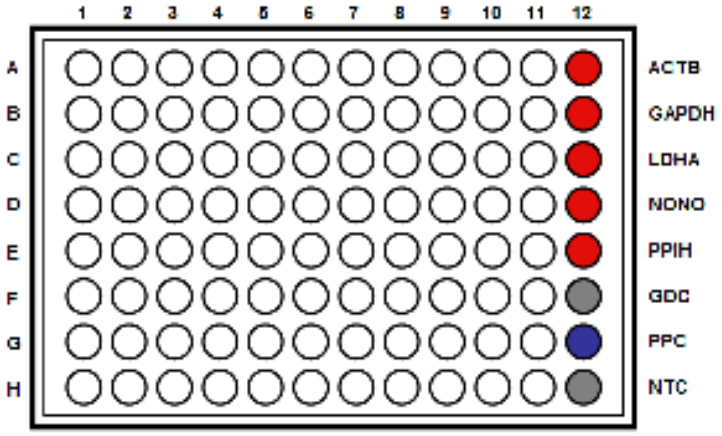


Table 2. Interpretation of control results:

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.

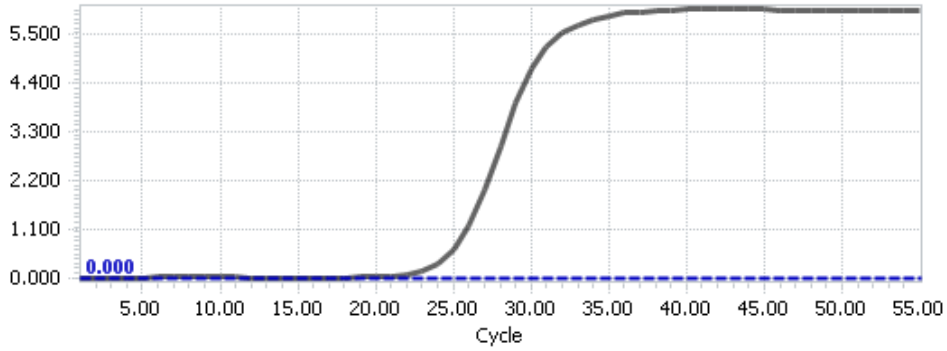
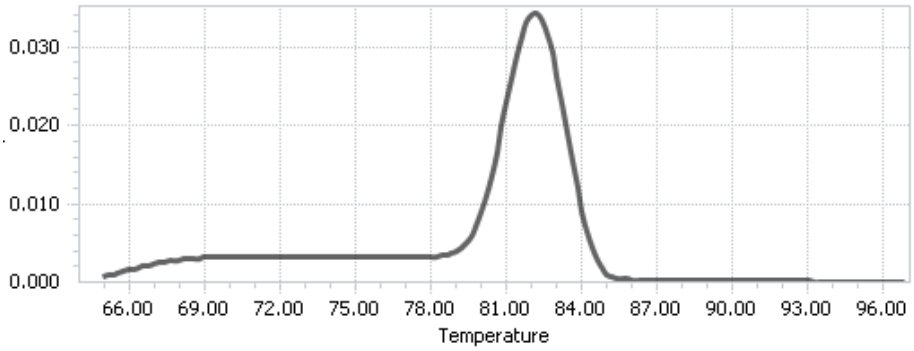


Figure 3. A typical melting peak of a qPCR product.



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 provide 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.

   $$\Delta Cq\ (ref) = average\ (\Delta Cq\ (HKG1), \Delta Cq\ (HKG2), \ldots\ldots, \Delta Cq\ (HKG\ n))$$ (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

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

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

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

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

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

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

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

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	ADAR	CCND1	DPYSL2	FOXA2	HRAS	IRAK1	MAPK8	NR4A1	PRKCA	RASA1	SOS1	***ACTB***
B	AKT1	CD14	EIF2AK2	FOXO1	HSPB1	IRS1	MDM2	PAK1	PRKCB	RBL2	SRF	***GAPDH***
C	AKT2	CDC42	EIF4B	FOXO3	HTT	ITGB1	MTOR	PDGFRA	PRKCZ	RHEB	TIRAP	***LDHA***
D	AKT3	CDKN1B	EIF4E	FOXO4	IGF1	JUN	MYD88	PDPK1	PTEN	RHOA	TLR4	***NONO***
E	APC	CHUK	ELK1	GJA1	IGF1R	MAP2K1	NFKB1	PIK3CA	PTK2	RPS6KA1	TSC1	***PPIH***
F	BAD	CREB1	FASLG	GRB2	IKBKB	MAPK1	NFKBIA	PIK3CG	PTPN11	RPS6KB1	TSC2	***GDC***
G	BTK	CSNK2A1	FKBP5	GSK3A	ILK	MAPK14	NOS1	PIK3R1	RAC1	RPS6KB2	WASL	***PPC***
H	CASP9	CTNNB1	FOS	GSK3B	INPP4B	MAPK3	NOS3	PIK3R2	RAF1	SHC1	YWHAH	***NTC***