Rev.1

GeneQuery™ Human Tie2/Angpt Signaling qPCR Array Kit (GQH-TEK)

Catalog #GK029

Product Description

ScienCell's GeneQuery™ Human Tie2/Angpt Signaling qPCR Array Kit (GQH-TEK) is designed to facilitate gene expression profiling of 88 key genes involved in Tie2 signaling transduction, which is a key component in the formation of blood vessels during angiogenesis. Brief examples of how included genes may be grouped according to function are shown below:

- **Ligands & receptors:** ANGPT1, ANGPT2, TEK, TIE1
- **Morphology:** GRB2, PTPN11, SOS1, PTK2, RHOA
- **Motility:** PLD1, MAP2K1, DOK2, NCK1, CRK
- **Transcription factors:** STAT1, STAT5B, MEF2A, FOXO3, EGR1
- **Cell cycle regulation:** CDC42, NFKB1, TNIP2, RIPK1, DIABLO

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	GK029	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 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-TEK 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.

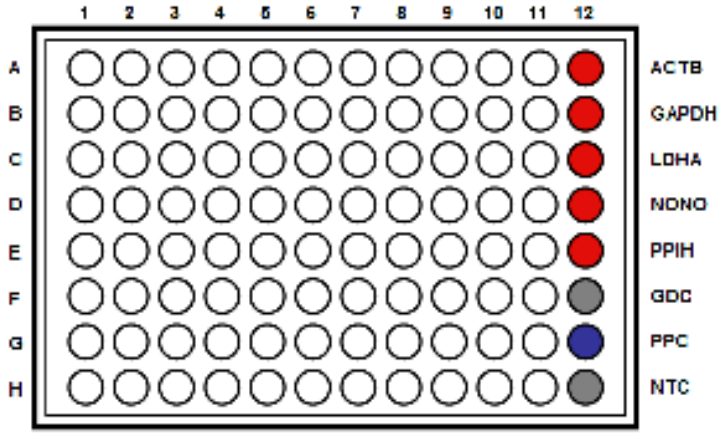


Table 2. Interpretation of control results:

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.

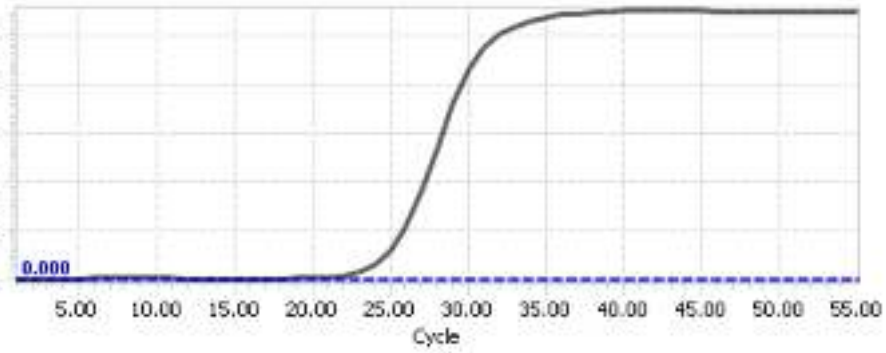
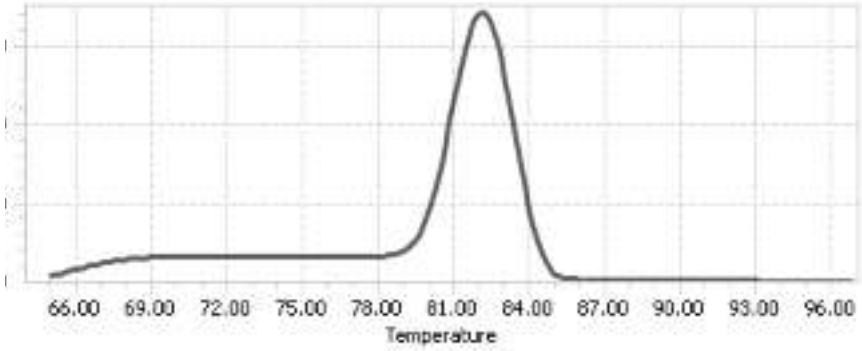


Figure 3. A typical melting peak of a qPCR product.



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

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

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

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

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

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

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 Tie2/Angpt Signaling qPCR Array Plate Layout* (***8 controls*** in Bold and Italic)

	1	2	3	4	5	6	7	8	9	10	11	12
A	ADSS	CDC42	DOK2	FOXO3	HIF1A	ITGB5	MMP2	PDIA3	PTPN11	SELE	TEK	***ACTB***
B	AKT1	CDKN1A	DOK4	FYN	HRAS	KLF2	MTOR	PGK1	PXN	SELP	TIAM1	***GAPDH***
C	AKT2	CRK	EGR1	GJA5	ICAM1	MAP2K1	NCK1	PIK3CA	RAC1	SOS1	TIE1	***LDHA***
D	ALDOA	CTNNB1	ELF2	GRB14	ILK	MAPK1	NFKB1	PIK3R1	RAF1	SPHK1	TLN1	***NONO***
E	ANGPT1	CXCL12	ELK1	GRB2	ITGA5	MAPK14	NOS3	PIK3R2	RASA1	STAT1	TNIP2	***PPIH***
F	ANGPT2	DIABLO	EPAS1	GRB7	ITGAV	MAPK3	NOX1	PLCG1	RELA	STAT3	TXNRD1	***GDC***
G	ARHGAP5	DIAPH1	FES	GYS1	ITGB1	MAPK7	NR4A1	PLD1	RHOA	STAT5A	VCAM1	***PPC***
H	BIRC5	DLL4	FOXO1	HAGH	ITGB3	MEF2A	PAK1	PTK2	RIPK1	STAT5B	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	GK029-A
	ABI 7000	GK029-A
	ABI 7300	GK029-A
	ABI 7500	GK029-A
	ABI 7700	GK029-A
	ABI 7900 HT	GK029-A
	QuantStudio	GK029-A
	ViiA 7	GK029-A
Bio-Rad	Chromo4	GK029-A
	iCycler	GK029-A
	iQ5	GK029-A
	MyiQ	GK029-A
	MyiQ2	GK029-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK029-A
	Matercycler ep realplex 4	GK029-A
Stratagene	MX3000P	GK029-A
	MX3005P	GK029-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK029-B
	ABI 7900 HT Fast	GK029-B
	QuantStudio Fast	GK029-B
	StepOnePlus	GK029-B
	ViiA 7 Fast	GK029-B
Bio-Rad	CFX Connect	GK029-B
	CFX96	GK029-B
	DNA Engine Opticon 2	GK029-B
Stratagene	MX4000	GK029-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK029-C
	Lightcycler 480 (96-well)	GK029-C