GeneQuery™ Human Trophoblast Cell Biology qPCR Array Kit (GQH-TRO)
Catalog #GK102

Product Description
Trophoblast cells form the outer layer of the blastocyst and subsequently differentiate into the intermediate trophoblast, cytotrophoblast, and syncytiotrophoblast layers. Trophoblasts play a central role in embryo implantation and interact with the maternal decidual layer of the placenta. ScienCell's GeneQuery™ Human Trophoblast Cell Biology qPCR Array Kit (GQH-TRO) is designed to facilitate gene expression profiling of 88 key genes involved in human trophoblast development, proliferation and differentiation, as well as important pathways in trophoblast cell biology, including LIF-STAT, FGF and NODAL/TGFB signaling pathways. Genes implicated in gestational trophoblastic disease and choriocarcinoma are also included in the array. Brief examples of how genes may be grouped according to their functions are shown below:

- **Trophoblast cell markers:** KRT7, GATA3, TFAP2C, ELF5, HLA-G, CGA, CGB3, CDX2, EOMES, ITGA1, VIM, POU5F1
- **Development and proliferation:** NANOG, SOX2, ESRRB, ASCL2, FLT1, HAND1, BHLHE40, GCM1, NODAL, PCSK6, FURIN
- **Trophoblast differentiation:** TCF4, ASCL2, ID1/2, TFAP2C, HAND1, THBD, EIF3A, NCOA6, HSF1, GCM1, DLX3
- **LIF-STAT signaling:** IL6ST, LIFR, STAT1/3, SOCS3, TIMP1, ITGB3, JUNB
- **FGF signaling:** FGF4, FGFR2, MAPK1/3, FRS2, PTPN11
- **NODAL/TGFB signaling:** NODAL, ACVR2A/B, ACVR1B, SMAD2/3
- **Gestational trophoblastic disease:** INHA, LNPEP, GPC3, EGFR, ERBB2/3, PAM, CGB3, KRT18, PRPTJ, ALPP
- **Choriocarcinoma:** HOPX, HLA-G, GH2, CGA, LNPEP, CYP19A1, ERV3-1, CYP11A1, ABCG2

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 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 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	Cat #	Quantity	Storage
GeneQuery™ array plate with lyophilized primers	GK102	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-TRO 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.

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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 H$_2$O 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	
Annealing	65°C	20 sec	40
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)	$Cq \geq 35$	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.)

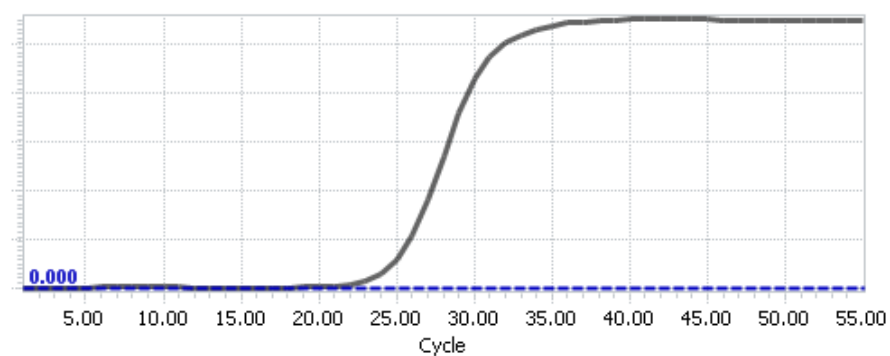Figure 2. A typical amplification curve showing the amplification of a qPCR product.
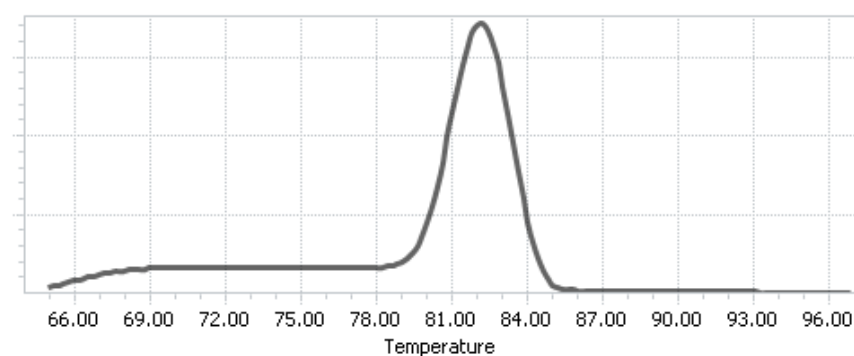

Figure 3. A typical melting peak of a qPCR product.

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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

$\quad\quad$ = ((17.16-18.20)+(17.84-18.48)+(20.12-20.57)+(19.64-19.50)+(26.40-26.55))/5

$\quad\quad$ = -0.43

ΔCq (GOI1) = 21.61 - 33.13

$\quad\quad\quad$ = -11.52

ΔCq (GOI2) = 22.19 - 26.47

$\quad\quad\quad$ = -4.28

$\Delta\Delta$Cq (GOI1) = ΔCq (GOI1) - ΔCq (ref)

$\quad\quad\quad$ = -11.52 - (-0.43)

$\quad\quad\quad$ = -11.09

$\Delta\Delta$Cq (GOI2) = ΔCq (GOI2) - ΔCq (ref)

$\quad\quad\quad$ = -4.28 - (-0.43)

$\quad\quad\quad$ = -3.85

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

$\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad$ = $2^{11.09}$

$\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad$ = 2180

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

$\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad$ = $2^{3.85}$

$\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad$ = 14.4

Conclusion: Upon treatment, expression level of GOI1 increased 2,180 fold, and expression level of GOI2 increased 14.4 fold.

ScienCell Research Laboratories

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GeneQuery™ Human Trophoblast Cell Biology qPCR Array Plate Layout*
(*8 controls* in Bold and Italic)

<u>*Note*</u>: all gene names follow their official symbols by HGNC

	1	2	3	4	5	6	7	8	9	10	11	12
A	ABCG2	BHLHE40	CYP19A1	ERV3-1	FURIN	HSF1	ITGB3	MAPK3	PAM	SLC22A4	TCF4	*ACTB*
B	ACVR1B	CCL2	DLX3	ERVW-1	GATA3	HSP90B1	JUNB	MGAT4A	PAPPA	SMAD2	TEAD3	*GAPDH*
C	ACVR2A	CD2AP	EGFR	ESRRB	GCM1	ID1	KHDC3L	NANOG	PCSK6	SMAD3	TFAP2A	*LDHA*
D	ACVR2B	CDKN1C	EIF3A	ETS2	GH2	ID2	KRT18	NCOA6	PLEKHH1	SMARCA4	TFAP2C	*NONO*
E	AFP	CDX2	ELF5	FGF4	GPC3	IGF2	KRT7	NLRP2	POU5F1	SOCS3	TFPI2	*PPIH*
F	ALPP	CGA	EOMES	FGFR2	HAND1	IL6ST	LIFR	NLRP5	PTPN11	SOX2	THBD	*GDC*
G	ALPPL2	CGB3	ERBB2	FLT1	HLA-G	INHA	LNPEP	NODAL	PTPRJ	STAT1	TIMP1	*PPC*
H	ASCL2	CYP11A1	ERBB3	FRS2	HOPX	ITGA1	MAPK1	NR0B1	SLC19A1	STAT3	VIM	*NTC*

* gene selection may be updated based on new research and development

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Appendix. Plate type choice chart.

Plate type A

Brand	Model	kit catalog #
ABI / Life Tech	ABI 5700	GK102-A
	ABI 7000	GK102-A
	ABI 7300	GK102-A
	ABI 7500	GK102-A
	ABI 7700	GK102-A
	ABI 7900 HT	GK102-A
	QuantStudio	GK102-A
	ViiA 7	GK102-A
Bio-Rad	Chromo4	GK102-A
	iCycler	GK102-A
	iQ5	GK102-A
	MyiQ	GK102-A
	MyiQ2	GK102-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK102-A
	Matercycler ep realplex 4	GK102-A
Stratagene	MX3000P	GK102-A
	MX3005P	GK102-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK102-B
	ABI 7900 HT Fast	GK102-B
	QuantStudio Fast	GK102-B
	StepOnePlus	GK102-B
	ViiA 7 Fast	GK102-B
Bio-Rad	CFX Connect	GK102-B
	CFX96	GK102-B
	DNA Engine Opticon 2	GK102-B
Stratagene	MX4000	GK102-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK102-C
	Lightcycler 480 (96-well)	GK102-C