



**GeneQuery™ Human Fibroblast Growth Factor Signaling Pathway
qPCR Array Kit
(GQH-FGF)
Catalog #GK040**

Product Description

ScienCell's GeneQuery™ Human Fibroblast Growth Factor Signaling Pathway qPCR Array Kit (GQH-FGF) is designed to facilitate gene expression profiling of 88 key genes involved in Fibroblast Growth Factor (FGF) signaling pathways. FGFs are involved in a wide variety of biological functions such as embryonic development, organogenesis, angiogenesis, tissue regeneration and wound healing. Through their binding with FGF receptors (FGFRs), FGFs activate and regulate many downstream signaling pathways, such as MAPK, JNK and STAT/NF-κB. Brief examples of how included genes may be grouped according to their functions are shown below:

- **Secreted FGFs**
 - **FGF1 superfamily:** FGF1, FGF2
 - **FGF4 superfamily:** FGF4, FGF5
 - **FGF7 superfamily:** FGF3, FGF7, FGF10
 - **FGF8 superfamily:** FGF17, FGF18
 - **FGF9 superfamily:** FGF9, FGF16, FGF20
- **Endocrine FGFs:** FGF19, FGF23
- **Intracellular FGFs:** FGF13, FGF14
- **FGF receptors:** FGFR1, FGFR2, FGFR3, FGFR5
- **FGF signaling inhibitors:** IL17RD, SPRY2, SPRY4, PTPN1, DUSP1, DUSP6
- **Activated downstream pathways**
 - **MAPK pathway:** FRS2, GAB1, SOS1, PTPN11, SHC1, RAS, BRAF, MAPK1, MAPK3, MAP2K1, MAP2K2
 - **PI3K/AKT pathway:** AKT1, AKT2, AKT3, PI3Ks, FOXO1, FOXO3, BAD, BAX, CASP9
 - **JNK pathway:** MAPK8, MAPK9, MAPK14, RAC1, ATF2, MAP2K4, MAP2K6
 - **STAT/NF-κB pathway:** STAT1, STAT3, NFκB1, NFκB2, REL, MMP9

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 Mg²⁺, 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 (β -actin, 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 | Quantity | Storage |
|---|----------|--------------|
| GeneQuery™ array plate with lyophilized primers | 1 | 4°C or -20°C |
| Optical PCR plate seal | 1 | RT |
| Nuclease-free H ₂ O | 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 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-FGF 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.

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 μ l PCR reactions for one well as shown in Table 1.

Table 1

| | |
|--------------------------------|-----------------------------|
| cDNA template | 0.2 – 250 ng |
| 2x qPCR master mix | 10 μ l |
| Nuclease-free H ₂ O | variable |
| <i>Total volume</i> | <i>20 μl</i> |

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 H₂O 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 | | |
| <i>Recommended</i> | <i>Melting curve analysis</i> | | 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.

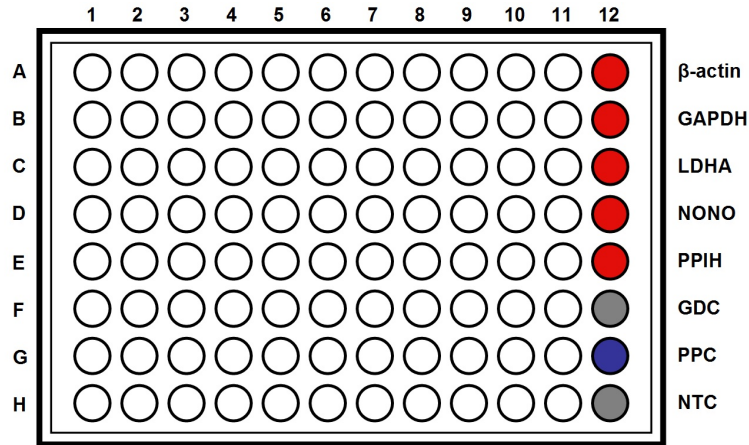


Table 2. Interpretation of control results:

| <i>Controls</i> | <i>Results</i> | <i>Interpretation</i> | <i>Suggestions</i> |
|----------------------------|--|--|--|
| 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.

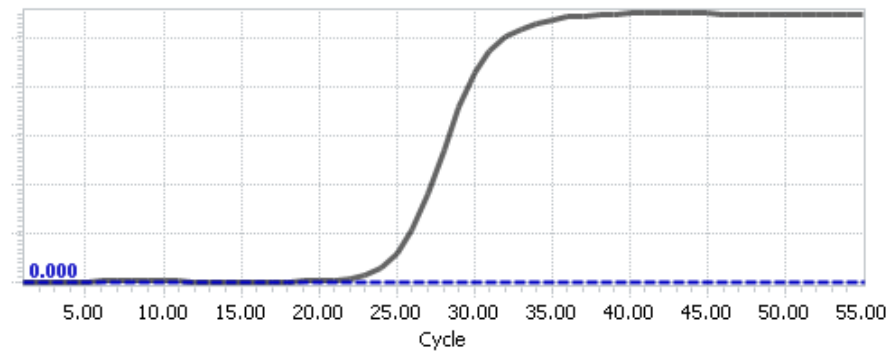
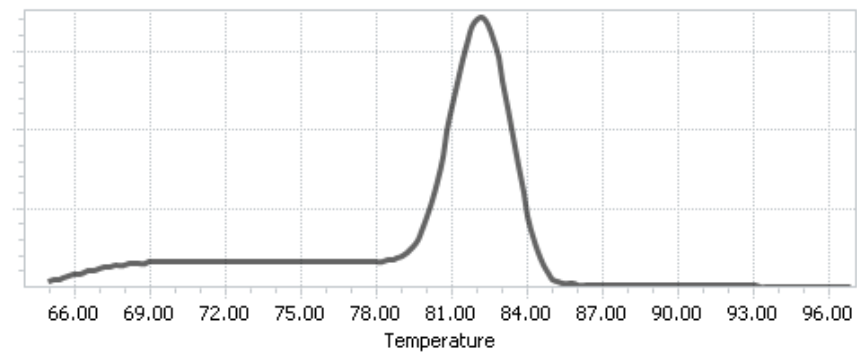


Figure 3. A typical melting peak of a qPCR product.



Quantification Method: Comparative $\Delta\Delta Cq$ (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.

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

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.

$\Delta Cq \text{ (ref)} = \text{average} (\Delta Cq \text{ (HKG1)}, \Delta Cq \text{ (HKG2)}, \dots, \Delta Cq \text{ (HKG n)})$ (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:

$$\Delta Cq \text{ (ref)} = (\Delta Cq(\beta\text{-actin}) + \Delta Cq(\text{GAPDH}) + \Delta Cq(\text{LDHA}) + \Delta Cq(\text{NONO}) + \Delta Cq(\text{PPIH})) / 5$$

Note: $\Delta Cq \text{ (HKG)} = Cq \text{ (HKG, experimental sample)} - Cq \text{ (HKG, control sample)}$, and $\Delta Cq \text{ (HKG)}$ value can be positive, 0, or negative.

3. For any of your genes of interest (GOI),

$$\Delta Cq \text{ (GOI)} = Cq \text{ (GOI, experimental sample)} - Cq \text{ (GOI, control sample)}$$

$$\Delta\Delta Cq = \Delta Cq \text{ (GOI)} - \Delta Cq \text{ (ref)}$$

$$\text{Normalized GOI expression level fold change} = 2^{-\Delta\Delta Cq}$$

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 $\Delta\Delta Cq$ (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.

| Samples | Genes of Interest | | Housekeeping Genes | | | | |
|--------------|-------------------|-------|---------------------------------|--------------|-------------|-------------|-------------|
| | GOI1 | GOI2 | <i>β-actin</i> | <i>GAPDH</i> | <i>LDHA</i> | <i>NONO</i> | <i>PPIH</i> |
| 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 |

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

$$\begin{aligned}\Delta Cq(\text{GOI1}) &= 21.61 - 33.13 \\ &= -11.52\end{aligned}$$

$$\begin{aligned}\Delta Cq(\text{GOI2}) &= 22.19 - 26.47 \\ &= -4.28\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq(\text{GOI1}) &= \Delta Cq(\text{GOI1}) - \Delta Cq(\text{ref}) \\ &= -11.52 - (-0.43) \\ &= -11.09\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq(\text{GOI2}) &= \Delta Cq(\text{GOI2}) - \Delta Cq(\text{ref}) \\ &= -4.28 - (-0.43) \\ &= -3.85\end{aligned}$$

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

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

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 Fibroblast Growth Factor Signaling Pathway qPCR Array Plate Layout*
(8 *controls* in Bold and Italic)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----------|-----------|-----------------------|
| A | AKT1 | DAG1 | FGF16 | FGF4 | FOS | IL17RD | MAPK14 | NFATC2 | PIK3CD | PLCG1 | SH2B1 | <i>β-actin</i> |
| B | AKT2 | DUSP1 | FGF17 | FGF5 | FOXO1 | JAK2 | MAPK3 | NFATC3 | PIK3CG | PTPN1 | SHC1 | <i>GAPDH</i> |
| C | AKT3 | DUSP6 | FGF18 | FGF7 | FOXO3 | KRAS | MAPK8 | NFATC4 | PIK3R1 | PTPN11 | SOS1 | <i>LDHA</i> |
| D | ATF2 | ELK1 | FGF19 | FGF9 | FOXO4 | MAP2K1 | MAPK9 | NFKB1 | PIK3R2 | RAC1 | SPRY2 | <i>NONO</i> |
| E | BAD | FGF1 | FGF2 | FGFR1 | FRS2 | MAP2K2 | MARCKS | NFKB2 | PIK3R3 | RAF1 | SPRY4 | <i>PPIH</i> |
| F | BAX | FGF10 | FGF20 | FGFR2 | GAB1 | MAP2K4 | MMP9 | NRAS | PIK3R4 | REL | SRC | <i>GDC</i> |
| G | BRAF | FGF13 | FGF23 | FGFR3 | GRB2 | MAP2K6 | NFAT5 | PIK3CA | PIK3R5 | RELA | STAT1 | <i>PPC</i> |
| H | CASP9 | FGF14 | FGF3 | FGFR5 | HRAS | MAPK1 | NFATC1 | PIK3CB | PIK3R6 | RELB | STAT3 | <i>NTC</i> |

* 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 | GK040-A |
| | ABI 7000 | GK040-A |
| | ABI 7300 | GK040-A |
| | ABI 7500 | GK040-A |
| | ABI 7700 | GK040-A |
| | ABI 7900 HT | GK040-A |
| | QuantStudio | GK040-A |
| | ViiA 7 | GK040-A |
| Bio-Rad | Chromo4 | GK040-A |
| | iCycler | GK040-A |
| | iQ5 | GK040-A |
| | MyiQ | GK040-A |
| | MyiQ2 | GK040-A |
| Eppendorf / Life Tech | Matercyler ep realplex 2 | GK040-A |
| | Matercyler ep realplex 4 | GK040-A |
| Stratagene | MX3000P | GK040-A |
| | MX3005P | GK040-A |

Plate type B

| Brand | Model | kit catalog # |
|-----------------|----------------------|---------------|
| ABI / Life Tech | ABI 7500 Fast | GK040-B |
| | ABI 7900 HT Fast | GK040-B |
| | QuantStudio Fast | GK040-B |
| | StepOnePlus | GK040-B |
| | ViiA 7 Fast | GK040-B |
| Bio-Rad | CFX Connect | GK040-B |
| | CFX96 | GK040-B |
| | DNA Engine Opticon 2 | GK040-B |
| Stratagene | MX4000 | GK040-B |

Plate type C

| Brand | Model | kit catalog # |
|-------|---------------------------|---------------|
| Roche | Lightcycler 96 | GK040-C |
| | Lightcycler 480 (96-well) | GK040-C |