# GeneQuery™ Human Transcription Factors qPCR Array Kit (GQH-TRF)

Catalog #GK124

## Product Description

ScienCell's GeneQuery™ Human Transcription Factors qPCR Array Kit (GQH-TRF) surveys a panel of 88 transcription factors. Transcription factors are proteins that can regulate target gene transcription level by binding to specific regions of genome known as enhancers or silencers. Misregulation of transcription factors can lead to aberrations in signaling transduction and a variety of disorders including autoimmunity, neurological disorders, diabetes, cardiovascular disease and cancer. Brief examples of how genes may be grouped according to their functions are shown below:

- **Stem cell-related:** NANOG, POU5F1, SOX2, EGR1, FOXD3, FOXO1, KLF4, MEF2B, PAX6, SOX18
- **Cancer stem cell-related:** NANOG, POU5F1, SOX2, AR, FOXO3, MYC, NKX3-1, SNAI1, SOX9, TFAP2A, TP53, TWIST1
- **Oxidative stress-related:** ARNT, ETS1/2, IRF1, JUN, NFAT5, NFKB1, NFYA, SMAD1, SRF, STAT3
- **Neuroprotective function-related:** CREB1, ESR1, FOXA1, GATA1/2/3, NFE2L2, NR4A2, PPARA, SP1, TBX3
- **Growth factor-mediated signaling pathways:** CREBBP, ELK1, FOS, HIF1A, ID1, NFAT5, NFATs, STAT1/3/5A
- **Wnt signaling pathway:** CEBPA, CEBPB, GBX2, HNF4A, LEF1, MITF, OLIG1, PITX2, SMAD4, SOX2
- **MAPK signaling pathway:** ATF2/4, ELK1, FOX, JUN, MAX, MEF2A/C, NFKB1, SP1, STAT1/3
- **B/T-cell receptor signaling pathways:** E2F1, ELK1, FOS, GATA3, JUN, MYB, NFATs, RUNX1, STAT6
- **Oncogenes & tumor suppressors:** ABL1, BRCA1, CDKN2A, JUNB, MYCN, NF1, RARA, RB1, TGFB1, TNF

***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** | **Quantity** | **Storage** |
|---|---|---|
| GeneQuery™ array plate with lyophilized primers | 1 | 4°C or -20°C |
| Optical PCR plate seal | 1 | RT |
| Nuclease-free $H_2O$ | 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-TRF 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 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.

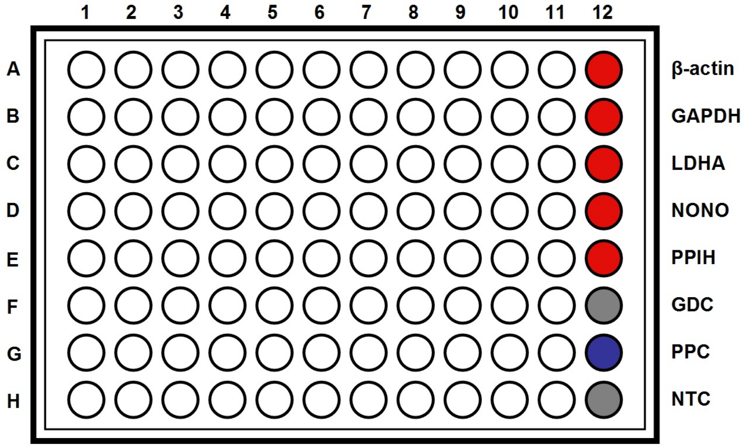


Table 2. Interpretation of control results:

| ***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<br>The Cq variations > 2 between qPCR Arrays. | Poor PCR performance; possible PCR inhibitor in reactions;<br>cycling program incorrect | Eliminate inhibitor by purifying samples;<br>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.

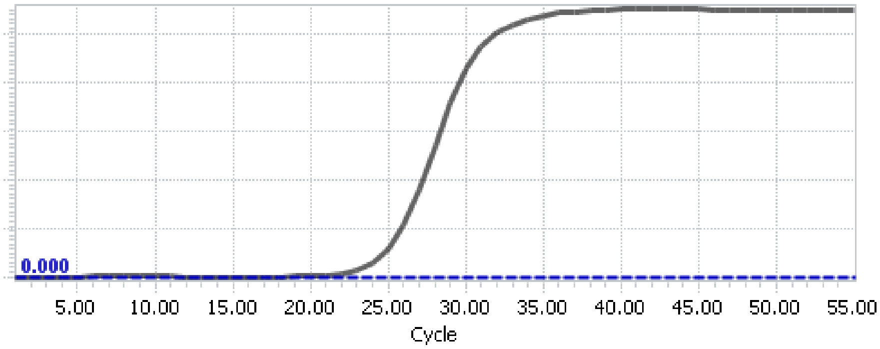
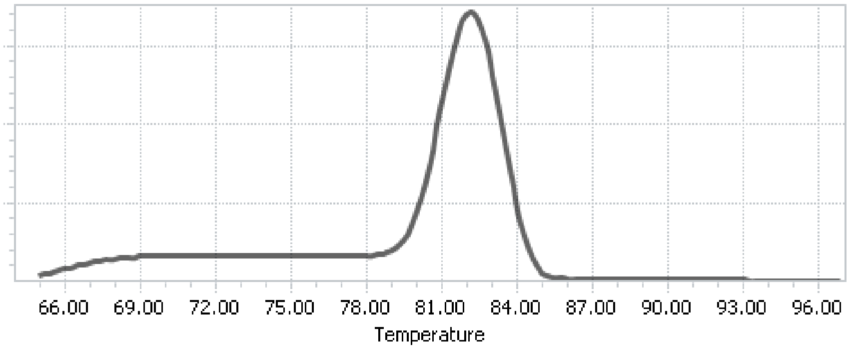


Figure 3. A typical melting peak of a qPCR product.



## 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, 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.

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

   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.

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

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

   ***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** | ***β-actin*** | ***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 |

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

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

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

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

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

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

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

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

ScienCell™
Research Laboratories

# GeneQuery™ Human Transcription Factors qPCR Array Kit (GQH-TRF)

Catalog #GK124

GeneQuery™ Human Transcription Factors qPCR Array Plate Layout*
(*8* ***controls*** in Bold and Italic)

***Note***: all gene names follow their official symbols by HGNC

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | ABL1 | CEBPB | ETS1 | GATA2 | IRF1 | MEF2C | NFATC1 | NR4A2 | REL | SOX18 | STAT6 | ***ACTB*** |
| **B** | AR | CREB1 | ETS2 | GATA3 | JUN | MITF | NFATC2 | OLIG1 | RELA | SOX2 | TBX3 | ***GAPDH*** |
| **C** | ARNT | CREBBP | FOS | GBX2 | JUNB | MYB | NFATC3 | PAX6 | RUNX1 | SOX9 | TFAP2A | ***LDHA*** |
| **D** | ATF2 | CTNNB1 | FOXA1 | GLI1 | KLF4 | MYC | NFATC4 | PITX2 | SMAD1 | SP1 | TGFB1 | ***NONO*** |
| **E** | ATF4 | E2F1 | FOXD3 | HIF1A | LEF1 | MYCN | NFE2L2 | POU5F1 | SMAD4 | SRF | TNF | ***PPIH*** |
| **F** | BRCA1 | EGR1 | FOXO1 | HNF4A | MAX | NANOG | NFKB1 | PPARA | SMAD5 | STAT1 | TP53 | ***GDC*** |
| **G** | CDKN2A | ELK1 | FOXO3 | HSF1 | MEF2A | NF1 | NFYA | RARA | SMAD9 | STAT3 | TWIST1 | ***PPC*** |
| **H** | CEBPA | ESR1 | GATA1 | ID1 | MEF2B | NFAT5 | NKX3-1 | RB1 | SNAI1 | STAT5A | WT1 | ***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 | GK124-A |
| | ABI 7000 | GK124-A |
| | ABI 7300 | GK124-A |
| | ABI 7500 | GK124-A |
| | ABI 7700 | GK124-A |
| | ABI 7900 HT | GK124-A |
| | QuantStudio | GK124-A |
| | ViiA 7 | GK124-A |
| Bio-Rad | Chromo4 | GK124-A |
| | iCycler | GK124-A |
| | iQ5 | GK124-A |
| | MyiQ | GK124-A |
| | MyiQ2 | GK124-A |
| Eppendorf / Life Tech | Matercycler ep realplex 2 | GK124-A |
| | Matercycler ep realplex 4 | GK124-A |
| Stratagene | MX3000P | GK124-A |
| | MX3005P | GK124-A |

### Plate type B

| Brand | Model | kit catalog # |
|---|---|---|
| ABI / Life Tech | ABI 7500 Fast | GK124-B |
| | ABI 7900 HT Fast | GK124-B |
| | QuantStudio Fast | GK124-B |
| | StepOnePlus | GK124-B |
| | ViiA 7 Fast | GK124-B |
| Bio-Rad | CFX Connect | GK124-B |
| | CFX96 | GK124-B |
| | DNA Engine Opticon 2 | GK124-B |
| Stratagene | MX4000 | GK124-B |

### Plate type C

| Brand | Model | kit catalog # |
|---|---|---|
| Roche | Lightcycler 96 | GK124-C |
| | Lightcycler 480 (96-well) | GK124-C |