GeneQuery™ Human Neural Development and Regeneration qPCR Array Kit (GQH-NDR)

Catalog #GK006

Product Description

ScienCell's GeneQuery™ human neural development and regeneration qPCR array kit (GQH-NDR) is designed to facilitate gene expression profiling of key genes involved in (i) human neurogenesis, migration and adhesion; (ii) neural development and differentiation; (iii) neural regeneration; as well as (iv) key genes in related degeneration diseases, such as Alzheimer's Parkinson's and Huntington's diseases. 88 genes are selected in this kit based on public database and literature research.

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 (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_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-NDR 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.

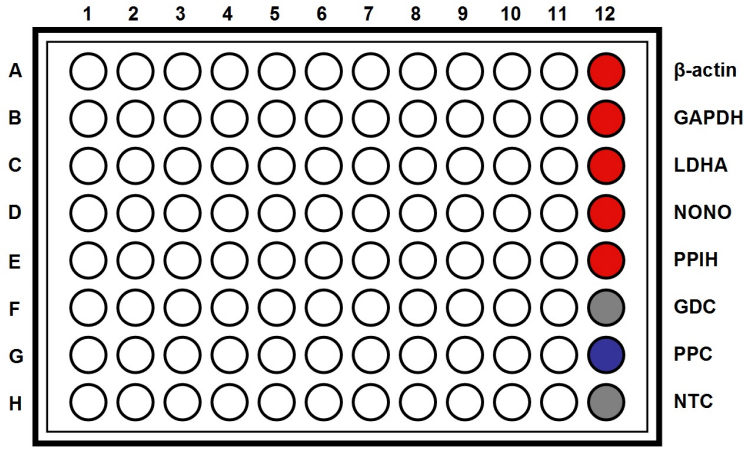


Table 2. Interpretation of control results:

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 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.

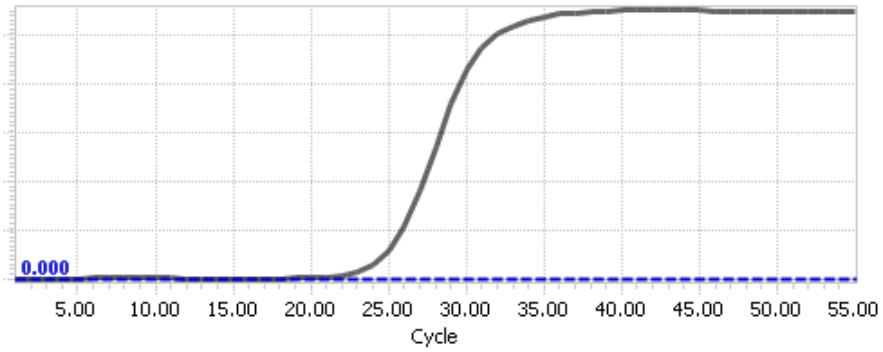
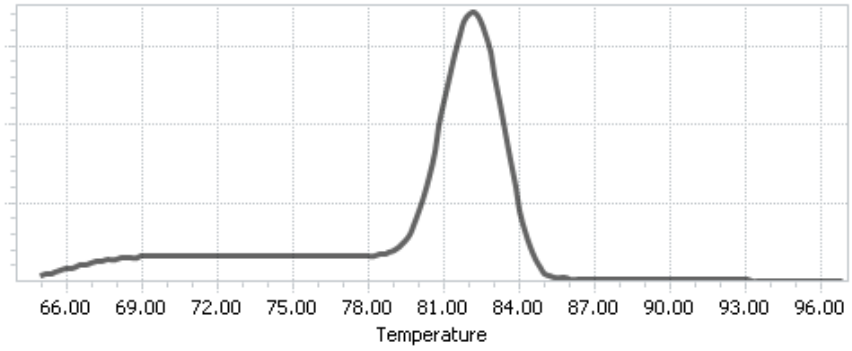


Figure 3. A typical melting peak of a qPCR product.



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GeneQuery™ Human Neural Development and Regeneration qPCR Array Plate Layout* (***8 controls*** in Bold and Italic)

	1	2	3	4	5	6	7	8	9	10	11	12
A	ABCA1	APP	BMP8B	DCX	GDNF	IL3	NEUROD1	NRP1	PARD3	POU4F1	S100B	***β-actin***
B	ABCA7	ARTN	CD33	DRD2	GPI	KMT2A	NEUROG1	NRP2	PARK2	PSEN1	SEPT5	***GAPDH***
C	ACHE	ASCL1	CDK5R1	EFNB1	HDAC4	LRP1	NEUROG2	NSG1	PARK7	PSEN2	SLC6A3	***LDHA***
D	ADORA1	BCL2	CDK5RAP2	EGF	HES1	MAP2	NOG	NTF3	PAX3	PTN	SLIT2	***NONO***
E	ADORA2A	BDNF	CLU	EP300	HEY1	MDK	NOTCH1	NTF4	PAX5	RAC1	SNCA	***PPIH***
F	APBB1	BIN1	CREB1	EPHA1	HEYL	MEF2C	NOTCH2	NTN1	PAX6	REST	SOX2	***GDC***
G	APBB2	BMP2	CREBBP	ERBB2	HTR2A	NDN	NR4A2	OLIG2	PICALM	ROBO1	TGFB1	***PPC***
H	APOE	BMP4	CXCL1	FGF2	HTT	NDP	NRCAM	PAFAH1B1	PINK1	S100A6	TNR	***NTC***

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