GeneQuery™ Human cDNA Evaluation Kit (GQH-CE)

Catalog #GK992

100 reactions

Product Description

ScienCell's GeneQuery™ Human cDNA Evaluation Kit (GQH-CE) assesses cDNA quality. The kit verifies successful reverse transcription of messenger RNA (mRNA) to complementary DNA (cDNA) and reveals the presence of genomic DNA (gDNA) contamination in cDNA samples. Good quality cDNA is a critical component for successful gene expression profiling. The GQH-CE kit is highly recommended for cDNA applications such as GeneQuery™ qPCR arrays.

Each primer set included in GQH-CE qPCR kit arrives lyophilized in a 2 mL vial. All primers are designed and tested under the same parameters: (i) an optimal annealing temperature of 65°C (with 2 mM Mg^{2+}, and no DMSO); (ii) recognition of all known target gene transcript variants; and (iii) specific amplification of only one amplicon. Each primer set has been validated by qPCR by melt curve analysis and gel electrophoresis.

GeneQuery™ Human cDNA Evaluation Kit Components

Cat. No.	Quantity	Component	Amplicon size
GK992a	1 vial	Human LDHA cDNA primer set (lyophilized, 100 reactions)	130 bp
GK992b	1 vial	Human genomic DNA Control (GDC) primer set (lyophilized, 100 reactions)	81 bp
GK992c	4 mL	Nuclease-free H_2O	N/A

- LDHA cDNA primer set targets housekeeping genes LDHA. The forward and reverse primers are located on different exons, giving variant amplicon sizes for cDNA and gDNA. For cDNA samples, LDHA primer set gives a 130 base pair (bp) PCR product.

- Genomic DNA Control (GDC) detects possible gDNA contamination in the cDNA samples. It contains a primer set targeting an 81 bp non-transcribed region of the genome on human chromosome 3.

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
Each primer set is validated by qPCR melt curve and amplification curve analyses. The PCR products are analyzed by gel electrophoresis to confirm single band amplification.

Product Use
GQH-CE 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 vials should be stored at 4°C and are good for up to 12 months. For long-term storage (>1 year), store the vials at -20°C in a manual defrost freezer.

Procedures

Note: The primers in each vial are lyophilized.

1. Prior to first use, allow vials to warm to room temperature.
2. Briefly centrifuge at 1,500x g for 1 minute.
3. Add 200 µl of nuclease-free H_2O to each vial to make primer stock solutions. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.
4. Prepare 20 µl PCR reactions for one well as shown in Table 1.

Table 1

Primer stock solution	2 µl
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.

5. Add the mixture of primer stock solution, cDNA template, 2x qPCR master mix, and nuclease-free H_2O to each well. Cap or seal the wells.
6. Briefly centrifuge the samples at 1,500x g for 1 minute at room temperature. For maximum reliability, replicates are recommended (minimum of 3).
7. 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:

Table 2. 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

8. (Optional) Load the PCR products on 1.5% agarose gel and perform electrophoresis to confirm the single band amplification in each well.

Appendix

Table 3. Interpretation of results:

Primers	***Results***	***Interpretation***	***Suggestions***
LDHA	Cq ≥ 35	There is no or very low cDNA content in the sample.	Optimize RNA extraction /reverse transcription procedure; make sure there is no nuclease presence in the system
gDNA Control (GDC)	Cq < 35	The sample is contaminated with gDNA	Optimize RNA extraction procedure
Positive PCR Control (PPC)	Cq > 30	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

Figure 1. A typical amplification curve showing the amplification of a qPCR product.

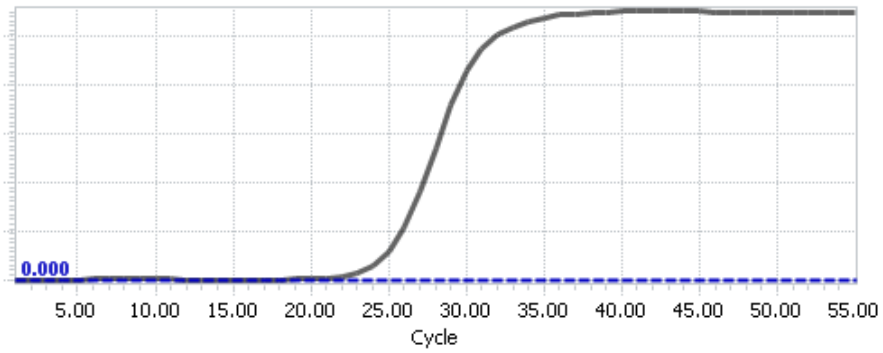

Figure 2. A typical melting peak of a qPCR product.