GeneQuery™ Rat cDNA Evaluation Kit (GQR-CE)

Catalog #GK992R

100 reactions

Product Description

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

Each primer set included in GQR-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™ Rat cDNA Evaluation Kit Components

Cat. No.	Quantity	Component	Amplicon size
GK992Ra	1 vial	Rat Actb cDNA primer set (lyophilized, 100 reactions)	93 bp
GK992Rb	1 vial	Rat genomic DNA control (RGDC) primer set (lyophilized, 100 reactions)	136 bp
GK992c	4 mL	Nuclease-free H_2O	N/A

- Rat Actb cDNA primer set targets rat housekeeping gene Actb. For rat cDNA samples, Actb primer set gives an 93 base pair (bp) PCR product.

- Rat Genomic DNA control (RGDC) detects possible rat gDNA contamination in the rat cDNA samples. It contains a primer set targeting a 136 bp non-transcribed region of the genome on rat chromosome 1.

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
GQR-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***
Actb	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 (RGDC)	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.

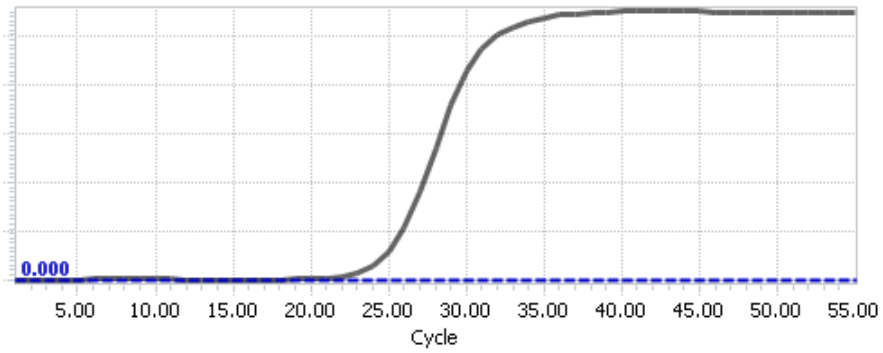

Figure 2. A typical melting peak of a qPCR product.