GeneQuery™ Mouse cDNA Evaluation Kit, Deluxe (GQM-CED)

Catalog #GK991M

100 reactions

Product Description

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

Each primer set included in GQM-CED 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™ Mouse cDNA Evaluation Kit, Deluxe Components

Cat. No.	Quantity	Component	Amplicon size
GK991Ma	1 vial	Mouse B2m cDNA primer set (lyophilized, 100 reactions)	86 bp
GK991Mb	1 vial	Mouse Gapdh cDNA primer set (lyophilized, 100 reactions)	135 bp
GK991Mc	1 vial	Mouse genomic DNA control (MGDC) primer set (lyophilized, 100 reactions)	93 bp
GK991d	1 vial	Positive PCR control (PPC) primer set (lyophilized, 100 reactions)	147 bp
GK991e	8 mL	Nuclease-free H_2O	N/A

- Mouse B2m cDNA primer set targets mouse housekeeping gene B2m. The forward and reverse primers are located on different exons, giving variant amplicon sizes for cDNA and gDNA. For mouse cDNA samples, B2m primer set gives an 86 base pair (bp) PCR product.

- Mouse Gapdh cDNA primer set targets mouse housekeeping gene Gapdh. The forward primer is located on an exon-exon junction, therefore mouse gDNA won't get amplified under suggested qPCR conditions listed in table 2. For mouse cDNA samples, Gapdh primer set gives a 135 bp PCR product.

- Mouse Genomic DNA Control (MGDC) detects possible gDNA contamination in the cDNA samples. It contains a primer set targeting a 93 bp non-transcribed region of the genome on mouse chromosome 5.

- 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 mouse genome, and thus tests the efficiency of the polymerase chain reaction itself.

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

GQM-CED 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***
B2m and Gapdh	Both 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 (MGDC)	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.

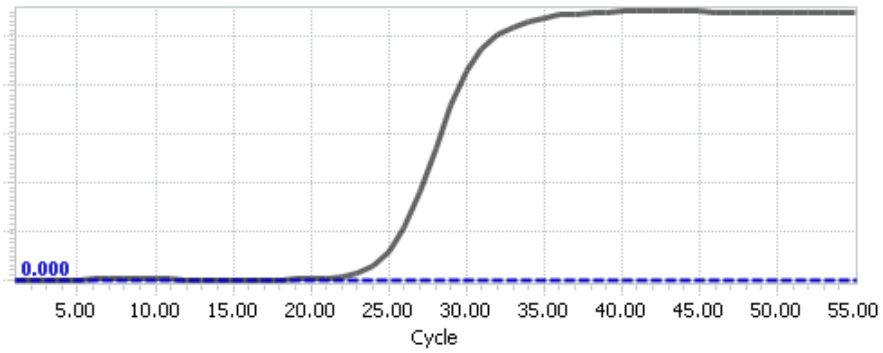

Figure 2. A typical melting peak of a qPCR product.