Human Mesenchymal Stem Cell Adipogenesis Detection qPCR Kit (HMSC-A-qPCR)

Catalog #8298

100 reactions

Product Description

Human mesenchymal stem cells (MSCs) are a population of multipotent cells that can be differentiated into multiple lineage-specific cells, which can form bone, fat, cartilage, muscle and tendon. Among them, adipocytes are one of the cell types that can be derived from MSCs through adipogenesis. The process of fat formation plays a role in obesity, cardiovascular diseases and metabolic disorders. ScienCell has created a convenient qPCR kit for the assessment of human mesenchymal stem cell adipogenesis. CEBPB, PPARG and FABP4 qPCR primers included in the kit allow for the detection and quantification of early-, mid- and late-stage human MSC adipogenesis, respectively.

***Note*:** all gene names follow their official symbols by the Human Genome Organization Gene Nomenclature Committee (HGNC).

Four qPCR controls are included in this kit to verify successful reverse transcription of messenger RNA (mRNA) to complementary DNA (cDNA), reveal the presence of genomic DNA (gDNA) contamination in cDNA samples, and detect qPCR inhibitor contamination. Good quality cDNA is a critical component for successful gene expression analysis.

Each primer set included in HMSC-A-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 Mg2+, 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, Deluxe Components

Cat. No.	Quantity	Component	Amplicon size
8298a	1 vial	Human CEBPB cDNA primer set (lyophilized, 100 reactions)	157 bp
8298b	1 vial	Human PPARG cDNA primer set (lyophilized, 100 reactions)	175 bp
8298c	1 vial	Human FABP4 cDNA primer set (lyophilized, 100 reactions)	113 bp
8298d	1 vial	Human housekeeping gene LDHA cDNA primer set (lyophilized, 100 reactions)	130 bp
8298e	1 vial	Human housekeeping gene PPIH cDNA primer set (lyophilized, 100 reactions)	149 bp

8298f	1 vial	Human genomic DNA Control (GDC) primer set (lyophilized, 100 reactions)	81 bp
8298g	1 vial	Positive PCR Control (PPC) primer set (lyophilized, 100 reactions)	147 bp
8298h	10 mL	Nuclease-free H_2O	N/A

- LDHA cDNA primer set targets housekeeping gene 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.
- PPIH cDNA primer set targets housekeeping gene PPIH. The forward and reverse primers of each set are located on different exons, giving variant amplicon sizes for cDNA and gDNA. For cDNA samples, PPIH primer set gives a 149 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.
- 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.

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

HMSC-A-qPCR kit 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 2 µM 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

2 µM primer stock	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 2 µM primer stock, 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:

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 2. Interpretation of results:

Primers	*Results*	*Interpretation*	*Suggestions*
LDHA and PPIH	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 (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.

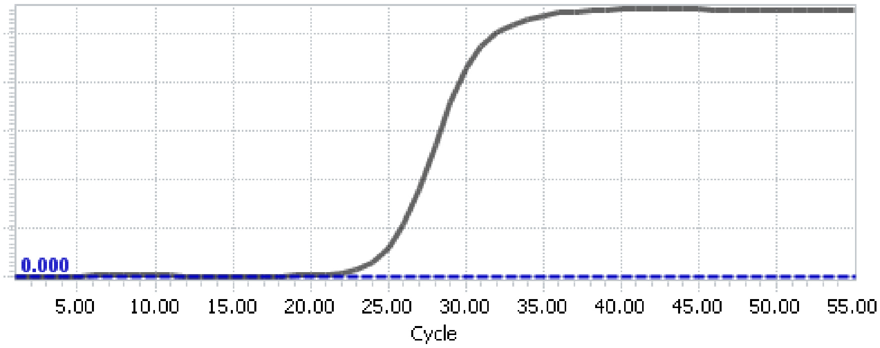
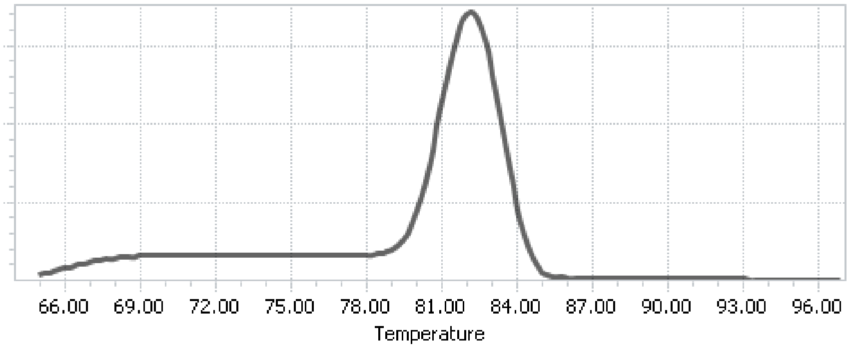


Figure 2. A typical melting peak of a qPCR product.



Quantification Method: Comparative $\Delta\Delta Cq$ (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 both housekeeping genes included in this kit, LDHA 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.

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

   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.

   ΔCq (ref) = average (ΔCq (HKG1), ΔCq (HKG2),......., ΔCq (HKG n)) (n is the number of housekeeping genes selected)

   If using both housekeeping genes included in this kit, LDHA and PPIH, use the following formula:

   $$\Delta Cq\ (\text{ref}) = (\Delta Cq(\text{LDHA})+\Delta Cq(\text{PPIH}))\ /2$$

   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),

   $$\Delta Cq\ (\text{GOI}) = Cq\ (\text{GOI, experimental sample}) - Cq\ (\text{GOI, control sample})$$

   $$\Delta\Delta Cq = \Delta Cq\ (\text{GOI}) - \Delta Cq\ (\text{ref})$$

   $$\text{Normalized GOI expression level fold change} = 2^{-\Delta\Delta Cq}$$

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 2 housekeeping genes obtained for experimental and control samples.

	Genes of Interest		Housekeeping Genes	
Samples	**GOI1**	**GOI2**	***LDHA***	***PPIH***
Experimental	21.61	22.19	20.12	26.40
Control	33.13	26.47	20.57	26.55

$\Delta Cq\ (ref) = (\Delta Cq(LDHA)+\Delta Cq(PPIH))\ /2$
$= ((20.12-20.57)+(26.40-26.55))/2$
$= -0.30$

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

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

$\Delta\Delta Cq\ (GOI1) = \Delta Cq\ (GOI1) - \Delta Cq\ (ref)$
$= -11.52 - (-0.30)$
$= -11.22$

$\Delta\Delta Cq\ (GOI2) = \Delta Cq\ (GOI2) - \Delta Cq\ (ref)$
$= -4.28 - (-0.30)$
$= -3.98$

Normalized GOI1 expression level fold change $= 2^{-\Delta\Delta Cq\ (GOI1)}$
$= 2^{11.22}$
$= 2385$

Normalized GOI2 expression level fold change $= 2^{-\Delta\Delta Cq\ (GOI2)}$
$= 2^{3.98}$
$= 15.8$

Conclusion: Upon treatment, expression level of GOI1 increased 2,385 fold, and expression level of GOI2 increased 15.8 fold.