Rev. 0

Human Telomeric repeat–containing RNA detection qPCR Kit (TERRA)

Catalog #9028
100 reactions

Product Description

Telomeric repeat–containing RNA (TERRA) is a long noncoding RNA transcribed from chromosome ends that plays a key role in telomere biology. TERRA molecules regulate telomerase activity, coordinate homologous recombination, influence telomere-associated protein complexes, and help maintain chromosome end integrity. Due to their low abundance and similarity to telomeric DNA, reliable detection of TERRA has historically been technically challenging.

The ScienCell Human Telomeric repeat–containing RNA detection qPCR Kit offers a sensitive and specific method for quantifying TERRA transcripts by RT-qPCR. The kit employs a proprietary DNA linker strategy during reverse transcription, which prevents amplification of contaminating genomic DNA during the qPCR step. This eliminates the need for harsh DNA removal procedures that can degrade TERRA and reduce assay accuracy. In the qPCR step, chromosome-specific subtelomeric forward primers are paired with linker–telomeric repeat reverse primers, enabling precise measurement of TERRA levels at individual chromosome ends. The GAPDH control is also included, with GAPDH specific forward and linker–GAPDH reverse primers, to serve as a housekeeping gene for normalization of gene expression analysis.

Kit Components

Cat #	Component	Quantity	Storage
9028a	TERRA cDNA Synthesis Master Mix, 4x	2 vials	-20°C
9028b	2q primer set	1 vial	-20°C
9028c	7p primer set	1 vial	-20°C
9028d	9p primer set	1 vial	-20°C
9028e	10q primer set	1 vial	-20°C
9028f	13q primer set	1 vial	-20°C
9028g	15q primer set	1 vial	-20°C
9028h	17p primer set	1 vial	-20°C
9028i	XqYq primer set	1 vial	-20°C
9028j	Total TERRA primer set	1 vial	-20°C
9028k	GAPDH primer set	1 vial	-20°C
9028l	Nuclease free water	4 ml	4°C

Additional Materials Required (Materials Not Included in Kit)

Component	Product Name
RNA isolation kit	DualPrep DNA/RNA Isolation Kit (ScienCell, Cat #MB6908)
RNA template	Customers' samples
2X qPCR master mix	GoldNStart TaqGreen qPCR, Master Mix (ScienCell, Cat. #MB6018)

Quality Control
Primer sets are validated by qPCR. cDNA amplification specificity is confirmed by the absence of amplification in reactions containing multiple concentrations of gDNA and in no-RT controls.

Product Use
TERRA 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 on dry ice. Upon receipt, store the nuclease-free H_2O (Cat #9028l) at 4°C and the rest of the kit at -20°C in a manual defrost freezer.

Procedures

Important: ***Only*** *use polymerases with hot-start capability to prevent possible primer-dimer formation.* ***Only*** *use nuclease-free reagents in PCR amplification.*

Reverse transcription:

1. Prior to use allow the TERRA cDNA Synthesis Master Mix (Cat#9028a) and RNA samples to thaw and place them on ice. Prepare the first strand synthesis reactions a shown in Table 1.

Table 1.

TERRA specific RT Master Mix, 4X (9028a)	10 µl
RNA template	5-10 µg
Nuclease-free H_2O (cat#9028l)	variable
Total volume	***20 µl***

2. For PCR program setup, follow Table 2.

Table 2.

Temperature	Time
25°C	10 min
45°C	60 min
85°C	10 min

3. Store the cDNA at -20°C for next step.

Quantitative Polymerase Chain Reaction (qPCR):

4. Prior to use, allow vials (Cat #9028b-#9028k) to warm to room temperature.

5. Centrifuge the vials at 1,500x g for 1 minute.

6. Add 200 µL nuclease-free H_2O (Cat #9028l) to each primer set (lyophilized, Cat #9028b-cat#9028k) to make primer stock solutions. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

7. For each cDNA sample prepare 10 qPCR reactions, one for each primer stock solution (cat #9028b-cat#9028k). Prepare 20 µL qPCR reactions for one well as shown in Table 1.

Table 1.

cDNA sample	2 µL
Primer stock solution (cat #9028b-cat#9028k)	2 µL
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µL
Nuclease-free H_2O (Cat #9018c)	6 µL
Total volume	***20 µL***

8. Seal the qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds. For maximum reliability, replicates are highly recommended (minimum of 3).

9. Refer to Table 3 for qPCR program setup. The 2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1) contains SYBR®Green as the reporter dye and does not contain a ROX passive reference dye. If the qPCR instrument being used has a "ROX passive reference dye" option, please deselect this option.

   Note: The primary factors that determine optimal annealing temperature are the primer length and primer composition. Based on the properties of primer sets (Cat #9028b-#9028k), we highly recommend an annealing temperature of 60°C as shown in Table 3:

Table 3.

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	20 sec	40
Annealing/Extension	60°C	60 sec	
Data acquisition	Plate read		

Note: *Since the amplified products for each subtelomere region vary in length due to differences in telomere repeat length, the melt curve may show multiple peaks, and the PCR products on the gel may appear as bands of different sizes.*

Quantification Method: Comparative ΔΔCq (Quantification Cycle Value) Method

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.

1. For 2q primer, ΔCq (2q) is the quantification cycle number difference of the 2q between the target and the reference cDNA samples.

   $$\Delta Cq\ (2q) = Cq\ (2q, \text{target sample}) - Cq\ (2q, \text{reference sample})$$

   Note: the value of ΔCq (2q) can be positive, 0, or negative.

2. For housekeeping, ΔCq (GAPDH) is the quantification cycle number difference of GAPDH between the target and the reference cDNA samples.

ΔCq (GAPDH) = Cq (GAPDH, target sample) - Cq (GAPDH, reference sample)

Note: the value of ΔCq (GAPDH) can be positive, 0, or negative.

3. $\Delta\Delta Cq = \Delta Cq$ (target primer) - ΔCq (GAPDH)
4. The Normalized 2q expression level fold change

$= 2^{-\Delta\Delta Cq}$

Note: This example applies to all other primers.

Example Calculations: Comparative $\Delta\Delta Cq$ (Quantification Cycle Value) Method

Table 3. Ct values from the qPCR of 2q and GAPDH primers using cDNA prepared from astrocytes treated with and without 400 μM H_2O_2 (Figure 1).

Primer set	*Target sample*	*Control sample*
2q	27.54	28.49
GAPDH	14.12	13.95

ΔCq (2q) = Cq (2q, target sample) - Cq (2q, reference sample)

= 27.54- 28.49

= - 0.95

ΔCq (GAPDH) = Cq (GAPDH, target sample) - Cq (GAPDH, reference sample)

= 14.12 – 13.95

= 0.17

$\Delta\Delta Cq = \Delta Cq$ (2q) - ΔCq (GAPDH)

= -0.95 – (0.17)

= -1.13

The expression level fold change of 2q subtelomric in the target sample to control sample

$= 2^{-\Delta\Delta Cq}$

$= 2^{-(-1.13)}$

= 2.18

Note: To measure total TERRA, use the Total TERRA primer set with GAPDH as a reference to determine relative changes in total TERRA expression.

Figure 1.

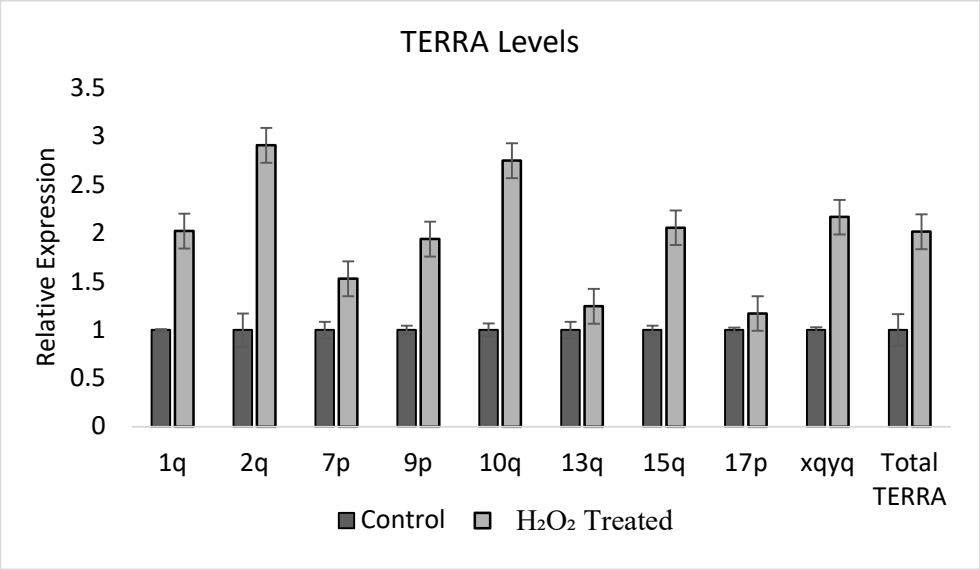

Figure 1. TERRAs are induced by oxidative stress in human astrocytes (cat#1800). Cells were incubated for 4 hours in the absence or presence of 400 µM H_2O_2, and TERRA expression was analyzed using the Human Telomeric Repeat–Containing RNA Detection qPCR Kit (cat#9028). Both total TERRA and TERRA originating from different subtelomeric sequences across various chromosome ends were measured and normalized to GAPDH.