Human Telomere Length Quantification qPCR Assay Kit

Cat #: orb1736804 (manual)

For research use only. Not intended for diagnostic use.

Cat.No	Specification	Storage/Shelf life
orb1736804 -01	100rxns	-20C°/one year
orb1736804 -02	500rxns	-20C°/one year

Advantages

- Get results quickly, saving 50% of time
- Optimized ready-to-use master mix for rapid PCR reactions.
- Accurate detection of various starting amounts of templates, stable amplification, and highly reproducible quantitative results.
- Balanced ration of $K^+$and $NH4^+$ ions, and independent ROX reference Dye packaging, suitable for all real-time PCR instruments.

Introduction

Telomere (Telomere) is a DNA sequence at the end of a eukaryotic cell chromosome composed of multiple repeating nucleotide elements (TTAGGG) in tandem. In addition to providing a buffer for non-transcribed DNA, it can also protect the end of the chromosome from fusion and Degenerate, protect chromosome structure stability and genetic integrity.

Telomere is the most important and accurate indicator of a person's aging rate. Its initial length is determined by genetic and environmental factors, and will decrease over time. Studies have shown that telomere length is closely related to DNA repair, aging, apoptosis, and tumorigenesis. Therefore, accurate and repeatable measurement of telomere length is particularly important for researchers. This product mainly uses relative quantitative qPCR to directly compare the average telomere length of the sample, that is, the copy number ratio (T/S) of the telomere repeat sequence (Tel) and the genome single copy gene (SCR) copy number (T/S) is used as the telomere relative length. Single-copy gene primers (SCR) specifically recognize and amplify the 78bp long region on human chromosome 11.

The primer set in the kit has passed the test to ensure: (1) efficient and reliable quantification; (2) no non-specific amplification. Each set of primers has been verified by amplification curve efficiency (E>98%, R2>0.99), melting curve analysis and gel electrophoresis verification.

Kit Components

Components	orb1736804 -01	orb1736804 -02
2× Mix	1ml	1.25mL×4

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Telomere Primer Mix (10uM)	50 µl	100 µl
Single Copy Reference Primer Mix (10uM)	50 µl	100 µl
50 x Rox	250 µl	1.25 ml
RNase-free ddH2O	1 ml	1.25mL×4
User manual	1 copy	1 copy

Principle

SYBR Green PCR SuperMix can perform specific and sensitive detection in a wide range, and is suitable for standard and rapid thermal cyclers. The SYBR Green I dye in the master mix can analyze multiple target nucleic acids without the need to synthesize sequence-specific probes. Antibody method hot-start Taq enzyme can effectively inhibit the amplification caused by primer non-specific annealing. At the same time, the PCR formula is optimized, suitable for the amplification of low-concentration templates, so that quantitative PCR can obtain a good standard curve in a wide quantitative area.

Kit application

This product is designed to confirm the average length of chromosomal telomeres in a sample by the ratio (T/S) of the copy number of telomere gene (Tel) and the copy number of single copy gene (SCR). It is for research use only and is not approved for clinical or in vitro diagnosis.

Attention

1.Template

Genomic DNA：0.5ng to 5ng of genomic DNA can be used in a 20µl system.

2.Transportation and storage methods

1）Ice pack, dry ice transportation.

2）Store at -20 °C and protect from light. This product contains the fluorescent dye SYBR Green I. When storing or preparing the reaction system, avoid strong light exposure. Be sure to invert and mix before use.

Reaction System

A reaction system as described below was established. To perform multiple reactions, prepare a premix of the common components, add a suitable volume to each tube or well, and then add a special reaction component (eg, template).

Composition	Volume (µL)	Final Concentration
2 x Mix	10µL	1 x
Primer stock solution（Telomere or SCR）	0.4µL	0.2µM
Genomic DNA Template（0.5~5ng/ul）	1µL	
*50 x ROX Dye (Optional)	0.4µL	1x

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RNase-free ddH2O	to 20μL	——

1. It is recommended to use a 20μL system to ensure the validity and repeatability of the amplification of the gene of interest.
2. Cover or seal the reaction tube/PCR plate and mix gently. It can be centrifuged slightly to ensure that all components are at the bottom of the tube.
3. Place the reaction system in a real-time PCR instrument, collect data and analyze the results. Set up your PCR instrument as shown in the table below. Optimum temperature

   The incubation time can be determined by the specific situation.

***ROX dye**

The fluorescent signal in the reaction system can be standardized by adding a ROX dye to the reaction system according to the selected instrument. The table below lists the amount of ROX required per unit of operation (per 50μL of reaction system):

Instrument	The amount of ROX required for each 50μL system reaction
ABI7300、7900HT、StepOne etc.	5μL
ABI7500、7500Fast、 ViiA7、Stratagene Mx3000™ 、Mx3005P™ and Mx4000™ etc.	1μL
Roche 、Bio-Rad， Eppendorf etc.	/

Three-Step amplification procedure:

Steps	Cycle number	Temperature	Time
Pre-denaturation	1x	95℃	1min
Denaturation	35-40x	95℃	10 sec
Annealing		55℃	30 sec
Extended		72℃	45 sec
Melt Curve			

Note: The main factors that determine the optimal annealing temperature are primer length and primer base composition. According to the characteristics of the kit's telomere and SCR primer set, we recommend setting the annealing temperature to 55℃.

Result analysis

At least three biological replicates are required for quantitative experiments. After the reaction is over, it is necessary to confirm the amplification curve and melting curve.