

Real Time Quantitative PCR

Cat.No.:SG00103

Description

Real-time quantitative PCR remains one of the most sensitive and quantitative tools for gene expression used today. Quantitative PCR(qPCR) is a powerful, highly sensitive technique that can be used to quantitate gene expression, determine gene copy number, detect SNPs, and detect DNA from viral and bacterial microorganisms. ShineGene support both SybrGreen and Taqman reaction chemistries. With Taqman chemistry, a labeled probe is included in the reaction to enhance the specificity and sensitivity of target sequence detection. With SyberGreen chemistry, amplified product is detected by its interaction with the SyberGreen fluorescent dye, which selectively binds to double-stranded DNA templates. We also offer primer and probe design services. We manage all aspects of the project from assay design to data analysis. Sample preparation (Nucleic acid isolation, whole genome amplification, etc.) is also available. You can get full service from ShineGene.

Application

Quantitative PCR can be applied in several key areas including:

- 1.Gene Expression Analysis
- 2.SNP Polymorphism Analysis
- 3.Quantification of miRNA
- 4.Drug Response Analysis
- 5.Cell Bank Copy Number Analysis
- 6.Mutation Analysis
- 7.Residual DNA Analysis



Reference

- 1.Chou, Q., Russell, M., Birch, D., Raymond, J. & Bloch, W. (1992) Prevention of pre-PCR mispriming and primer dimerization improves low-copy-number amplifications. *Nucleic Acids Res.*20:1717–1723.
- 2.Roux, K. H. (1995) Optimization and troubleshooting in PCR. *PCR Methods Appl.* 4:5185–5194.
- 3.Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(t)) Method.*Methods* 2001, 25:402-408.
- 4.L D Ke, Z Chen .A reliability test of standard-based quantitative PCR: exogenous vs endogenous standards *Mol Cell Probes.* 2000 Apr;14(2):127-35.
- 5.Weihong Liu and David A. Saint Validation of a quantitative method for real time PCR kinetics *Biochemical and Biophysical Research Communications* 294 (2002) 347–353
- 6.Heid, C.A., et al. Real time quantitative PCR. *Genome Research* 6(10), 986-94, 2002.
- 7.Pusterla, N., et al. Quantitative real-time PCR for detection of members of the Ehrlichia phagocytophila genogroup in host animals and Ixodes ricinus ticks. *J. Clin. Microbiol.* 37(5), 1329-31, 1999.

Price List

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Full service (from sample to data acquisition) You can get full service from ShineGene. E-mail: master@shinegene.org.cn

RNA Isolation from Sample	20.00\$/sample
cDNA Synthesis	5.00\$/reaction
qPCR	15.00/reaction
Taqman Probe	150.00\$/probe
Sybr Green I	be free

Time

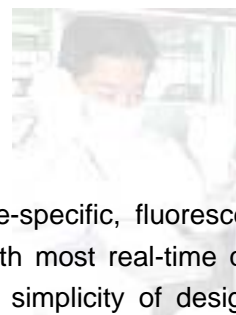
The turn around time from sample to data is about 30 working days.

Sample Requirements

1. Cells (in vitro cultured cells or white blood cells, purified peripheral blood mononuclear cells (PBMCs): One to 5 million cells. When FACS-sorted and highly purified cells are provided, the number may be as low as 100,000 cells. We have performed TaqMan® analysis for gene transcription using blastocysts at the 32 and 64-cell stage with excellent results. The enough harvested cells which be stored in Trizol® could be shipped frozen (-20°C) in styrofoam boxes. The styrofoam boxes should be recycled.

2. Tissues: Fresh tissue should be provided in a frozen state on dry ice (stored at -80°C for up to 1 year). Please provide tissue samples at least 50-100 mg. The grinded sample which be stored in Trizol® could be shipped frozen (-20°C) in styrofoam boxes. The styrofoam boxes should be recycled.

3. Fixed tissues: Two 50 micron sections of paraffin-embedded tissues are required from solid tissues. A third 50 micron section is required from non-solid tissues such as lung tissue. TaqMan® analysis were performed on a variety of paraffin-embedded tissues (i.e. skin, lymph node, GI tract, spleen, liver, lung, brain) which were up to 20 years of age without significant loss of PCR signals for the endogenous control (GAPDH, beta-actin or 18S rRNA) when compared to recently embedded tissue (within 1 year).



Fluorogenic Probes Label

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Dual-labeled fluorogenic probes are highly-sensitive, sequence-specific, fluorescent probes designed for real-time quantitative PCR. They can be used with most real-time quantitative PCR instruments and multiplex analysis systems due to their simplicity of design and the extensive range of fluorophores available.

Labels for Taqman/Beacon fluorogenic probes

5'Reporters	3'Quenchers
6-FAM, HEX, TET, ROX, Cy3, Cy5, Texas Red, Rhodamine	TAMRA, Dabcyl, BHQ™-1 or BHQ-2

1. Deprotected, desalted and purified by PAGE or RP-HPLC
2. Available in lengths of 15 to 40 mers
3. Delivered dried in individual, opaque tubes
4. Shipped within 7 to 8 working days of receiving your order, pending successful QC validation

Price

Name	2 OD (\$)	5 OD (\$)	Time
Taqman(5'Fam,3'Tamra)	150.00/ probe	180.00 / probe	7working days
Beacon(5'Fam,3'Dabcyl)	150.00/ probe	180.00 / probe	7working days

Note: Estimate 1 OD = 5 nmols = 33 µg, for a 20 mer oligo



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