Pre-Developed TaqMan® Assay Reagents Control Kits

Insert P/N 4319902 REV B
Printed in USA

For Research Use Only.
Not for use in diagnostic procedures.

Control kit contents:
Control primers and probe reagent

Notice to Purchaser: Disclaimer of License

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This Pre-Developed TaqMan® Assay Reagents Control Kit is covered by U.S. Patent 5,723,591 and foreign counterparts and patents pending owned by Applied Biosystems, as well as U.S. Patents 5,801,155 and 6,084,102 and foreign counterparts licensed to Applied Biosystems.

Overview

Introduction

Applied Biosystems developed the Pre-Developed TaqMan® Assay Reagents product as a research tool for Real Time, *in vitro* relative quantitative evaluation of gene expression. The Pre-Developed TaqMan® Assay Reagents detect the expression of target sequences in complementary DNA (cDNA) samples. The procedure outlined in the Pre-Developed TaqMan® Assay Reagents Gene Quantification Protocol (P/N 4310255) describes how to evaluate gene expression using Applied Biosystems Sequence Detection Systems. It is strongly recommended to read this protocol before using the Pre-Developed TaqMan® Assay Reagents. For further information on relative gene expression, see Bulletin #2 Relative Quantitative on Gene Expression (P/N 4303859).

Guidelines

Although the Pre-Developed TaqMan® Assay Reagents are designed for the general assay of cDNA samples, they do have some recommendations for use.

Please consider the following information before proceeding:

- Reverse transcription of total RNA to cDNA for gene expression assays should always be done with random hexamers. Use the TaqMan® Reverse Transcription Reagents (P/N N808-0234) for this step.
- Multiplex PCR will not work on the GeneAmp® 5700 Sequence Detection System. Thus, target and control reactions must be performed in separate tubes when used with this system.

Materials

This product contains sufficient control primers and probe to detect the control sequence. The control reagent is optimized for use with TaqMan® Universal PCR Master Mix to perform 1000 PCR reactions (50 µL each) but does not contain TaqMan® Universal PCR Master Mix. The following Applied Biosystems products are for use with this product:

TaqMan® Universal PCR Master Mix (P/N 4304437). The mix is optimized for the TaqMan® reaction and contains AmpliTaq Gold® DNA Polymerase, AmpErase® UNG, dNTPs with dUTP, Passive Reference, and optimized buffer components.

Applied Biosystems Sequence Detection System
Sequence Detection Systems Spectral Calibration Kit (P/N 4305822)
MicroAmp® Optical 96-well reaction plate/Optical Caps (P/N 403012)
TaqMan® Reverse Transcription Reagents (P/N N808-0234)

Storage and Stability

Upon receipt, store the 20X Pre-Developed TaqMan® Assay Reagents at -15°C to –25°C.

Preparing the PCR Reaction Mix

The preparation of the reaction mix used in the following tables is crucial for the accurate calculation of relative quantification values. The first table should be used for control only PCR reactions while the second table should be used for multiplexed (target plus control) PCR reactions. Prepare the PCR reaction mixture for each sample in a separate microcentrifuge tube before aliquoting the samples to the reaction plate for thermal cycling and fluorescence analysis. The volume of PCR reaction mix per well must be 50 µL minus the volume of the cDNA sample. Load 10 ng to 1 µg of cDNA (converted from total RNA) per well, depending on your target of interest. The contents of the PCR reaction mix are as follows.

Components (Control Only Reactions) Concentration	Volume/Tube (µL)	Final
RNase-free water	22.5 µL – y	-
2X TaqMan® Universal PCR Master Mix	25 µL	1X
20X Target Primers and Probe	2.5 µL	1X
cDNA sample	y (µL)	-
Total Volume	50 µL	1X

Components (Multiplex; Target + Control Reactions) Concentration	Volume/Tube (µL)	Final
Rnase-free water	20 µL – y	-
2X TaqMan® Universal PCR Master Mix	25 µL	1X
20X Target Primers and Probe	2.5 µL	1X
20X Control Primers and Probe	2.5 µL	1X
cDNA sample	y (µL)	-
Total Volume	50 µL	1X

Note : This reaction is optimized for TaqMan® Universal PCR Master Mix. Mix the PCR reaction mixture and cDNA samples prior to addition to the MicroAmp® Optical 96 Well Reaction Plate to ensure optimal performance of your PCR reactions.

Setting Thermal Cycler Conditions

Times and Temperatures

	Initial Steps		Each of 40 cycles	
			Melt	Anneal/Extend
Stage	Hold	Hold		Cycle
Temperature	50.0°C	95.0°C	95.0°C	60.0°C
Time (min.)	2:00	10:00	00:15	1:00

Important: The 2-minute, 50.0C step is required for optimal AmpErase® UNG enzyme activity. The 10 minute, 95.0C step is required to activate AmpliTaq Gold® DNA Polymerase.

Ordering Information

For Research use only. Not for use in Diagnostic procedures.

In the United States:

To order Pre-Developed TaqMan® Assay Reagents please
FAX a completed order form. To receive an order form, contact your sales representative at
FAX: 650-638-5998

Product Insert
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