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Assays-on-Demand™ Gene Expression Products**Product Insert****P/N: 4331182****Overview**

Assays-on-Demand Gene Expression Products (part number 4331182) consist of a 20X mix of unlabeled PCR primers and TaqMan® MGB probe (FAM™ dye-labeled). These assays are designed for the detection and quantification of specific human genetic sequences in RNA samples converted to cDNA. Gene expression quantification using Assays-on-Demand Products is performed in the second step of a two-step reverse transcription-polymerase chain reaction (RT-PCR) protocol on any ABI PRISM® Sequence Detection Systems instrument. All Assays-on-Demand Gene Expression Products are optimized to work with either TaqMan® Universal PCR Master Mix, No AmpErase® UNG (P/N 4324018) or TaqMan® Universal PCR Master Mix (P/N 4304437) and with complementary DNA (cDNA). These products utilize the universal thermal cycling parameters described below in Table 2.

Procedure

To prepare the reaction components for a single 20µL reaction (384-Well Clear Optical Reaction Plate) or a single 50µL reaction (96-Well Optical reaction Plate) refer to Table 1 for singleplex reactions.

Table 1. Singleplex PCR Reaction Mix using TaqMan® Universal PCR Master Mix, No AmpErase UNG (P/N 4324018)

Reaction Components	Volume/Well 20µL volume reaction ¹	Volume /Well 50µL volume reaction ¹	Final Concentration
TaqMan® Universal PCR Master Mix, No AmpErase® UNG (2X) ² 20X Assays-on Demand	10	25	1X
Gene Expression Product Target	1	2.5	1X
cDNA diluted in RNase-free water	9	22.5	--
Total	20	50	

1. If different reaction volumes are used, amounts should be adjusted accordingly
2. Volumes should be the same if using TaqMan® Universal PCR Master Mix (2X) P/N (4304437)

Table 2. Thermal Cycler Conditions

Thermal Cycler	Times and Temperature			
	Initial Setup		Each of 40 Cycles	
	HOLD ³	HOLD	Denature	Anneal/Extend
Sequence Detection System Instrumentation	UNG Activation		CYCLE	
	2 min 50°C	10 min 95°C	15 sec 95°C	1 min 60°C

3. The two-minute, 50°C step is required for optimal AmpErase® UNG activity when using TaqMan® Universal PCR Master Mix P/N (4304437). This step is not needed when using TaqMan® Universal PCR Master Mix, No AmpErase® UNG P/N (4324018)

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For further information on the plate set-up procedure and data analysis refer to the User's Manual for the appropriate Sequence Detection System Instruments.(7900HT,7000,7700)

Gene expression using Assays-on-Demand Products should be performed in separate wells (singleplex assay). We recommend that the endogenous control of choice is run in separate wells (singleplex) as this does not require carrying out any validation experiments. If performing multiplex* experiments, we recommend that an experiment is run in which a multiplex versus singleplex assay is performed to confirm that the Ct values are not affected by multiplex PCR amplification.

For additional information regarding relative quantitation of gene expression experiments refer to the ABI PRISM® 7700 Sequence Detection System User Bulletin #2 (P/N 4303859).

*Multiplex PCR is the use of more than one primer pair in the same tube. Refer to the ABI PRISM® Sequence Detection System User Bulletin #5 (P/N 4306236) for information regarding multiplex reactions.

Storage:

Store between -15°C and -20°C, and minimize freeze thaw cycles. The 20X assay mix may be diluted in TE (final concentration of TE should be 10mM Tris-HCL/1mM EDTA pH 8.0, use RNase free water).

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

Notice to purchaser: Limited License

TaqMan® probes are covered by U.S. Patent 5,723,591 and foreign counterparts and patents pending owned by PE Corporation (NY), and may be covered by U.S. Patents 5,801,155 and 6,084,102 and foreign counterparts licensed to Applied Biosystems.

Notice to Purchaser: Disclaimer of License

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