

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 quantitation of specific human genetic sequences in RNA samples converted to cDNA. Gene expression quantitation using Assays-on-Demand™ products is performed as the second step in a two-step reverse transcription-polymerase chain reaction (RT-PCR) protocol on any ABI PRISM® Sequence Detection System 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 plate) or a single 50µL reaction (96-well plate) refer to Table 1 below for singleplex reactions.

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

Reaction Component	Volume/Well (20µL volume reaction ¹)	Volume/Well (50µL volume reaction ¹)	Final Concentration
TaqMan® Universal PCR Master Mix, No AmpErase® UNG (2X) ²	10	25	1X
20X Assays-on-Demand™ Gene Expression Assay Mix	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 Temperatures			
	Initial Setup		Each of 40 Cycles	
			Denature	Anneal/Extend
Sequence Detection Systems (7900HT, 7700, 7000)	HOLD ³	HOLD	CYCLE	
	UNG activation 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 the TaqMan® Universal PCR Master Mix, No AmpErase® UNG (P/N 4324018).

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, 7700, 7000).

Gene expression using Assays-on-Demand™ products should be performed in separate wells (singleplex assay). We recommend that the endogenous control of choice be run in separate wells (singleplex) as this does not require any validation experiments. If performing multiplex* experiments, we recommend running multiplex and singleplex assays in parallel to confirm that the C_T 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; minimize freeze thaw cycles. The 20X Assays-on-Demand™ Gene Expression 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

This product is optimized for use in the Polymerase Chain Reaction (PCR) and 5' nuclease detection methods covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd. No license under these patents to use the PCR process or 5' nuclease detection methods is conveyed expressly or by implication to the purchaser by the purchase of this product. A license to use the PCR process for certain research and development activities accompanies the purchase of certain Applied Biosystems reagents when used in conjunction with an authorized thermal cycler, or is available from Applied Biosystems. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501, USA.