A.B.T.™ 2X qPCR Probe MasterMix (without UDG)

Cat#	Size
Q01-01-01	1 ml
Q01-01-05	5 ml

Description: A.B.T.™ 2X qPCR Probe MasterMix (without UDG) is suitable for most of quantitative Real-time PCR. The MasterMix solution is ready for use. A hot start Taq DNA Polymerase (antibody mediated), dNTPs, enhancer, $MgCl_2$ and stabilizer are the components of the MasterMix. A.B.T.™ 2X qPCR Probe MasterMix (without UDG) is highly compatible with fluorescent labeled probes (e.g. Taqman probes). A.B.T.™ 2X qPCR Probe MasterMix (without UDG) is suitable for real-time PCR, gene knockdown verification, gene expression profiling and array validation applications. DNA samples such as cDNA, genomic DNA and plasmid DNA are strongly amplified and distinguished with A.B.T.™ 2X qPCR Probe MasterMix (without UDG).

Kit Components:

Components	Q01-01-01	Q01-01-05
A.B.T.™ 2X qPCR Probe MasterMix (without UDG)	1 ml	5 ml
ROX Dye (50X)	50 µl	250 µl

Recommended Protocol: All reagents should be thawed on ice, gently mixed and briefly centrifuged before use. This protocol is exemplary for one reaction, and for multiple reactions it is necessary to calculate the components in a proportional manner. Prepare each of following components, combine on ice and place in heated (95°C) thermal cycler:

For 20 µl PCR Reaction	Volume	Final Conc.
A.B.T.™ 2X qPCR Probe MasterMix (without UDG)	10 µl	1X
ROX Dye (50X) (Optional)	0.4 µl (0.04µl)	1X (0.1X)
Forward Primer (10 µM)	0.2 - 2 µl	0.1 - 1 µM
Reverse Primer (10 µM)	0.2 - 2 µl	0.1 - 1 µM
Fluorescence Labeled Probe	Variable	0.1 - 1 µM
Template	Variable	Variable
RNase-Free Distilled Water	up to 20 µl	-

Note: For each 50 µl reaction use 0.6 - 1.0 µl ROX Dye (Final concentration: 300 - 500 nM) in devices requiring high ROX concentration (ABI 7000, 7300, 7700, 7900HT and 7900HT Fast, StepOne, StepOne Plus).
For each 50 µl reaction use 0.06 - 0.1 µl ROX Dye (Final concentration: 30 - 50 nM) in devices requiring low ROX concentration (ABI 7500, 7500 Fast, Viia 7, QuantStudio; Roche LightCycler; Stratagene, Mx3000, Mx3005P and Mx4000).

General Cycling Conditions:

PCR Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	300 sec.	1
Denature	95	10 - 30 sec.	25 - 40
Anneal	55 - 68	10 - 60 sec.	

Notice: Depending on different primer and template combinations cycling conditions can be optimized. For example, raise the annealing temperature to prevent non-specific primer binding. On the other hand, increase extension time to generate longer PCR products.

Storage Conditions: Store all contents at -20ºC in a freezer. After thawing the MasterMix store at 4°C for up to 6 months, without showing any reduction in performance.

Quality Control: Precision and reproducibility controlled in reactions involving dilutions of a 10-fold nucleic acid template in real-time PCR.

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