BIOATLAS

Science of Life

Atlas Probe 1-step RT-qPCR Kit

Cat. No.	Pack Size
BH40801	100 rxn
BH40802	500 rxn

Shipping and Storage conditions:

Shipping and storage at -20ºC.

Application:

- Detection and quantification of RNA targets with qPCR
- DNA/LNA hydrolysis probe-based assays
- Suitable for singleplex or duplex reactions

Kit contents:

Atlas Probe 1-step RT-qPCR Kit contains all the components necessary (except template, primers and probes) to perform cDNA synthesis and probe-based qPCR in a single tube.

- **Atlas Probe 1-step Enzyme Mix (10X)**
- ***Atlas Probe 1-step Reaction Buffer (2X)***
- ***Nuclease-free Water***
- ***Low ROX (50X)***
- ***High ROX (50X)***

Description:

Atlas Probe 1-step RT-qPCR Kit is a high-quality premix based on probes for 1-step quantitative reverse transcription PCR (RT-qPCR), which is mainly used for specific ultra-high sensitivity quantitative detection of RNA.

Atlas 1-step RT-qPCR Kit uses the extracted RNA as a template and uses qPCR primers to carry out reverse transcription and fluorescence quantitative PCR continuously in the same tube. It is easy to operate, which minimizes human errors, effectively reduces the risk of contamination, saves the operation time of the PCR experiment, and has large detection throughput.

Detection Principle:

Probe-based qPCR uses fluorescent and quencher-labeled DNA probes to target the sequence which will be amplified by PCR. Normally, quenching groups on the probe result in quenching of fluorescent groups due to the fluorescence resonance energy transfer (FRET) in space. When the target sequence is amplified by PCR reaction, both primers and probes are annealed to the target gene. With the extension of primers, the 5' to 3' exonuclease activity of Taq enzyme will cause the probe bound to the target sequence to be degraded gradually from the 5' end. After the fluorescent group and quenching group of the probe are cleaved by Taq enzyme, the quenching group disappears, and the fluorescent group can be normally excited by the excitation light to produce fluorescence. After each PCR cycle, more fluorescent groups are released, and the fluorescence intensity is proportional to the number of newly synthesized target fragments, thus quantitative detection can be achieved. Probes are usually a fragment of linear DNA specific to the target sequence, labeled with fluorescent groups such as FAM or HEX at the 5' end and fluorescent quenching groups such as BHQ1, TAMRA or MGB at the 3' end.

Features:

- High specificity and sensitivity: specificity is not only dependent on PCR primers, but also specific binding and degradation of probes and target genes to generate fluorescent signals. The detection sensitivity and specificity are usually significantly higher than those of the methods using fluorescent dyes.
- Multiple detection: in a single reaction, different genes correspond to different probes and different probes correspond to different fluorescent markers, which can be used for multiple fluorescent quantitative PCR detection. Atlas Probe 1-step RT-qPCR mix can be used for the detection of 2-3 genes at the same time after optimization of primers and probes.

ROX Dye and normalization:

Atlas Probe 1-step RT-qPCR Kit provides Low ROX and High ROX dye.
ROX is an internal passive reference dye used to normalize the fluorescent reporter signal generated in qPCR.
Atlas Probe 1-step RT-qPCR Kit components are s**uitable for ROX-dependent and ROX-independent qPCR cyclers.**

Protocol:

1. Mix the reagents required for the Probe 1-step RT-qPCR reaction and keep them on ice.

2. Set up Probe 1-step RT-qPCR reaction as the following table

Recommended set up 1-step RT-qPCR reaction:

Components	Volume	Final conc.
Atlas Probe 1-step Reaction Buffer (2X)	10 µl	1 x
Atlas Probe 1-step Enzyme Mix (10X)	2 µl	1 x
Primers (forward and reverse) - 3 µM each	2 µl	300 nM
Probe	variable	200 nM
Template RNA	variable	1 pg-2 µg
With or without Low/High **ROX** (50X)	0.4 µl	1 x
Nuclease- free H_2O	Up to 20 µl	
Total reaction volume	20 µl	

Mix the reagents required for the Probe 1-step RT-qPRC reactions on ice.

3. Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin.

4. Transfer PCR tubes from ice to a PCR machine.

Recommended thermocycling conditions for 1-step RT-qPCR:

Cycle step	Temp.	Time	Cycles
Reverse transcription	**50ºC**	**15-30 min**	1
Initial denaturation	**95ºC**	**2 min**	1
Denaturation	95ºC	15 s	40
Annealing/Elongation	60ºC	15-30 s	
Melting curve analysis (optional)	**95ºC** **60ºC** **95ºC**	15 sec 15 sec 15 sec	

Safety warnings and precautions:

For *in vitro* use only

This product and its components should be handled only by persons trained in laboratory techniques. It is advisable to wear suitable protective clothing, such as laboratory overalls, gloves and safety glasses. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.
Some applications this product is used in may require a license which is not provided by the purchase of this product. Users should obtain the license if required.

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