One-Step RT-qPCR Kit

Catalogue No.:abx460027

Abbexa's One-Step RT-qPCR Kit provides high sensitivity of the target RNA level due to its reverse transcriptase and RNase inhibitors mix which aim to diminish RNA degradation and mispriming during reaction setup and reverse transcription to guarantee optimal RT efficiency. The kit also contains a 2X concentrated ready-to-use universal qPCR Master Mix, optimized for probe-based real-time PCR and compatible with the majority of commercially available real-time PCR systems (ROX-independent and ROX-dependent). It contains antibody-mediated hot-start Taq DNA polymerase, dNTPs, $MgCl_2$, enhancers, stabilizers and essentials for a successful PCR reaction.

Kit Components (100 rxns):

- 2X Universal qPCR Master Mix: 1 ml
- Enzyme Mix: 20 µl

Target:	One-Step RT-qPCR Kit
Tested Applications:	RT-PCR
Storage:	Store at 4 °C for up to 3 months. For long-term storage, store at -20 °C. Avoid repeated freeze/thaw cycles.
Buffer:	Contains 30-50 mM Tris-HCl, 0.3-0.5 mg/ml BSA and < 0.3% Triton X-100.
Note:	THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

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Directions for use: Assay Procedure:

1. Bring all reaction components to 4 °C and ensure all vials are thawed. Briefly centrifuge the vials before opening to ensure complete recovery of vial contents. Avoid exposure to light.
2. Add components to a PCR tube on ice or at room temperature according to the table below. Adjust the volumes accordingly for different reaction volumes. The concentration of primers may require optimization.

Component	Volume
2X Reaction Mix	10 µl
Enzyme Mix	0.2 µl
Forward and Reverse Primers	Variable Recommended Final Concentration: 300 nM each
Fluorogenic Probe(s)	Variable Recommended Final Concentration: 150-250 nM each
RNA Template	Variable Total RNA: 1 ng - 5 µg
Nuclease-Free Water	Variable, up to 20 µl
Total Reaction Volume	**20 µl**

3. Mix by gently pipetting up and down.
4. Close the lid on the tube and vortex for at least 30 seconds.
5. Briefly centrifuge the tubes to remove any air bubbles.
6. Set up the thermal cycling conditions according to the table below. Optimization may be required. Load the PCR tubes into the PCR instrument and commence the run.

Number of Cycles	Stage	Temperature	Time per Cycle
1 cycle	cDNA Synthesis	42 °C	15 min
1 cycle	Pre-Denaturation	95 °C	5 min
35-45 cycles	Denaturation	95 °C	10 seconds
	Annealing	60 °C	60 seconds
1 cycle	Instrument Cooling	40 °C	10 seconds

7. Perform data analysis according to the instrument's manual.

Notes:

• Avoid cross-contamination with DNA by cleaning workstation areas with 10% bleach rather than ethanol, as this will hydrolyze and dissolve any residual DNA.
• Poor or no signal may be observed if inhibitors are present, the template has degraded, or the thermal cycling extension time is insufficient.
• High primer concentrations may result in primer dimers.
• A thermal gradient can be carried out to determine the optimal thermal cycling conditions for a specific primer set.

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