Product Information	
StableScript™ One-Step RT-qPCR Kit	
Part Number	P7730L
Storage Temperature	-25ºC to -15ºC
Reaction Size	250 Reactions
Volume	0.5 mL
Lot Number	
Reference Number	

Product Description:
The StableScript One-Step RT-qPCR kit is a highly efficient and sensitive RT-qPCR formulation for detection using hydrolysis probes. It supplied with a 4X reaction buffer that contains dNTP's and Magnesium. The 10X enzyme mix contains a blend of hot start taq, a new RNAse H minus reverse transcriptase with higher thermostability (StableScript) and specific enhancers to increase sensitivity and inhibitor resistance.

Product Specifications	
Assay	**RT-qPCR**
Specification	Amplification of Test Lot within 1Ct of Reference Lot in a one-step RT-qPCR assay

Quality Control Analysis:
The functionality of the RT-PCR Assay is evaluated by amplification of three mRNA transcripts in a one-step RT-qPCR assay. The amplification threshold (Ct) of the test lot is compared to a reference lot.

Notes:
Enzyme components were tested prior to formulation of the master mix and found free of contaminating endonucleases and exonucleases. Enzyme purity was >99% as determined by SDS-PAGE and negligible *E.coli* genomic DNA contamination was confirmed by qPCR. Specific activity was verified for each enzyme pre-formulation.

Supplied with:
StableScript™ Reaction Buffer (4X) B7720

Related Products:
Thermolabile UDG G5020L
StableScript™ P7720L

Limitations of Use
This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.

Protocol

General precautions against degradation of RNA template should be taken when setting up a reaction, including setting up the reaction with nuclease free water, RNase inhibitor, nuclease free PCR tubes and sterile pipette tips with filter, adding reverse transcriptase last, gentle pipetting, thorough mixing and a quick centrifugation. The following procedure can be used as a guideline. Reactions may need to be optimized individually.

1. The 4X StableScript Reaction Buffer should be thaw completely and vortex for 3-5 seconds to mix thoroughly. Quickly spin to collect contents.
2. Prepare primer/probe mix. A final concentration of 0.4-0.9µM for each primer and 0.1-0.5µM for probe are recommended. However, the optimal concentration for primers/probe needs to be empirically determined for each assay.
3. Determine the number of reactions to prepare, including No Template Controls (NTCs). Add 10% extra volume to compensate for the pipetting loss.
4. Follow the table below to set up the reaction mix. It is recommended to make a master mix to minimize variations and potential errors.

Components	Volume/Rxn	Final Concentration
4X StableScript Reaction Buffer	5µL	1X
Primer/Probe Mix	XµL	Variable
10X StableScript Enzyme Mix	2µL	1X
RNA Template	Up to 2µL	1pg to 1µg total RNA
Nuclease-Free Water	To a final **20µL** reaction volume	-

5. Seal the PCR plate and spin briefly to collect contents at the bottom.

Thermal Cycling Conditions:

Program the cycling conditions are recommended as below.

Standard Cycling Program* Steps	Temperature	Time	Cycles
Reverse Transcription	55°C	10 min	1
Taq Activation/Initial Denaturation	95°C	3 min	1
Denaturation	95°C	5-10 sec	40
Annealing/Extension*	60°C	30-60 sec	

*Cycling parameters can be modified (especially the annealing/extension condition) to fit specific assays.

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