

Product Information				
ZipScript™ WarmX™ One-Step RT-qPCR Mix				
Part Number	Y9460L			
Storage Temperature	-25ºC to -15ºC			
Lot Number				
Reaction Size	250 Reactions			
Reference Number				

## **Product Description:**

The 10X ZipScript™ WarmX One-Step RT-qPCR Mix is a highly sensitive and reproducible RT-qPCR solution optimized for real-time PCR. The aptamer based warm start feature of RT reduces non-specific reverse transcription during reaction setup and improves assay specificity and consistency. The 10X enzyme mix is accompanied by a 2X reaction buffer.

Product Specifications  Y9460					
Assay	RT-qPCR	Warm Start Function			
Specification	Amplification of Test Lot within 1Ct of Reference Lot in a one- step RT-qPCR Assay.	Melting curve analysis confirmed that no non- specific amplification is detected.			

#### **Quality Control Analysis:**

#### **Functional Assay**

The functionality of the ZipScript™ WarmX 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.

#### Function Assay of the warm start mechanism

The warm start feature of ZipScript™ WarmX is tested by SYBR-based RT-qPCR amplification of an mRNA transcript after a 24-hour pre-incubation of the reaction at 25°C. Melting curve analysis confirmed that no non-specific amplification is detected.

## 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:

ZipScript™ Reaction Buffer I (2X) B7641: 2 vials, 1.5ml each

#### **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. SDS sheets relevant to this product are available upon request.



## **Recommended Protocol**

## ZipScript™ WarmX One-Step RT-qPCR Reaction Setup:

- 1. Thaw the 2X ZipScript™ Reaction Buffer I completely and vortex for 3-5 seconds to mix thoroughly. Quick spin to collect contents if necessary.
- 2. Prepare primer/probe mix. A final concentration of 0.4- $0.9 \mu M$  for each primer and 0.1- $0.5 \mu M$  for probe is 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 Conc
RNA Template	Up to 2 μL	-
2X ZipScript™ Reaction Buffer I (B7641)	10 μL	1X
Primer/Probe Mix	Primer/Probe Mix X μL	
10X ZipScript™ WarmX Enzyme Mix	2 μL	1X
Nuclease-Free Water  To a final 20 μL reaction volume		-

5. Seal PCR plate and spin briefly to bring down reagents.

# **Thermal Cycling Conditions:**

Program the cycling conditions based on the recommendations below.

Standard Cycling Program\*

Steps	Temperature	Time	Cycles	
Reverse Transcription	50°C	15 min	1	
Taq Activation/Initial Denaturation	95°C	2 min	1	
Denaturation	95°C	15 sec	40	
Annealing/Extension*	60°C	30-60 sec	.0	

<sup>\*</sup> Cycling parameters can be modified (especially the annealing/extension condition) to fit specific primer/probe selection.

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