Azura 1-Step Ultra RT-PCR Kit

Catalog No.	Pack Size	Kit Components	
AZ-1825	50 reactions	2x Azura 1-Step Ultra Mix	20x RT Enzyme Blend
AZ-1826	100 reactions	2x Azura 1-Step Ultra Mix	20x RT Enzyme Blend

Description

Azura 1-Step Ultra RT-PCR Kit has been formulated for cDNA synthesis and subsequent PCR in a single tube for end-point analysis. This new generation RT-PCR Kit consists of a thermostable Reverse Transcriptase, a potent RNase Inhibitor and Azura HS Taq for ultra-sensitive one-step RT-PCR from as little as 1pg total RNA starting material. The advanced and highly optimized buffer chemistry allows for efficient reverse transcription and PCR of problematic sequences with significant secondary structure (GC-rich targets). The Azura 1-Step Ultra RT-PCR Kit is ideal for determining the presence or absence of RNA templates and quantifying expression through qualitative analysis of RNA transcription levels. The kit also efficiently synthesizes double-stranded cDNA for subsequent gene expression analysis.

- **Sensitive**: Optimized chemistry for detection of low-copy transcripts
- **Robust:** Overcomes secondary structure in problematic GC-rich targets
- **Convenient**: First strand full-length cDNA synthesis and PCR in a single tube.

Storage

Azura 1-Step Ultra RT-PCR Kit is shipped on blue or dry ice and should be stored at –20°C upon receipt. Excessive freeze/thawing should be avoided.

Important Guidelines

2x Azura 1-Step Ultra Mix: The 2x Mix includes HS Taq Polymerase, 2 mM dNTPs, 6 mM $MgCl_2$, and PCR enhancers for maximum efficiency, sensitivity and success with difficult amplicons. We do not suggest the use of additional PCR enhancers.

20x RT Enzyme Blend: This is a highly optimized 20x concentration of thermostable reverse transcriptase and RNase inhibitor.

Template: Use 1 pg to 1 µg total RNA per reaction (or a minimum of 0.01 pg mRNA per reaction).

Reverse Transcription: We recommend an incubation temperature of 50°C for 10 minutes.

Primers: Primers should have a predicted melting temperature of around 60°C, using default Primer 3 settings (http://frodo.wi.mit.edu/primer3/). The final primer concentration in the reaction should be between 0.2 µM and 0.6 µM.

Annealing: We recommend performing a temperature gradient to determine the optimal annealing temperature. Alternatively, 60°C can be used as a starting point. For optimization, increase in 2°C increments. For example, if non-specific products are present or smearing is visible, a higher annealing temperature is required.

Extension: Optimal extension is achieved at 72°C. The optimal extension time is dependent on amplicon length and complexity. 30 seconds per kilobase (kb) is recommended for amplification from eukaryotic genomic DNA or cDNA up to 3 kb.

Reaction setup

1. Prepare a PCR master mix based on following table (mix reagents well by inversion and centrifuge briefly):

Component	50 µl Reaction	Final Concentration/Notes
2x 1-Step Ultra Mix	25 µl	1X
Forward Primer (10 µM)	2.0 µl	400 nM
Reverse Primer (10 µM)	2.0 µl	400 nM
20x RT Enzyme Blend	2.5 µl	1x
Template RNA	1 pg to 1 µg Total RNA	>0.01 pg mRNA
PCR-grade water	Up to 50 µl final volume	

2. Reverse Transcription and PCR cycling:

Cycles	Temperature & Time	Notes
1	50°C, 10 minutes	Ideal for a wide range of assays
1	95°C, 2 minutes	Initial Denaturation, polymerase activation
30 - 40	95°C, 15 seconds 55°C to 65°C, 15 seconds 72°C, 30 seconds per kb	Denaturation Annealing (determined by user) Extension

Quality Control

Azura 1-Step Ultra RT-PCR Kit is tested extensively for robust activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. Azura 1-Step Ultra RT-PCR Kit is manufactured under a comprehensive quality management system, following ISO 9001:2015 standards.

Limitations of Use

This product is intended for research purposes only and is not intended for any animal or human therapeutic use.

Technical Support

For Trouble-shooting and Technical Guidance, please contact us at tech@azuragenomics.com and provide PCR reaction conditions, cycling parameters, amplicon size, and screen grabs (gel images) if possible.