

Azura One-Step Ultra RT-PCR Kit

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

Description

Azura One-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 One-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 from 1pg total RNA.

Storage

Azura One-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. When stored as specified, Azura One-Step Ultra RT-PCR Kit is stable for 12 months from date of receipt. The Kit may also be stored at 4°C for 1 month.

Important Guidelines

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

1-Step Enzyme Blend: The highly optimized blend of thermostable RTase, RNase inhibitor and Azura HS Taq DNA Polymerase.

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

Reverse Transcription: We recommend an incubation temperature of 45°C for 15 minutes. However, for regions of high secondary structure, incubation temperatures up to 50°C may be used. For amplicons above 1kb, the incubation time should be increased to 25 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 68°C . The optimal extension time is dependent on amplicon length and complexity. 60 seconds per kilobase(Kb) is recommended for amplification from eukaryotic genomic DNA or cDNA up to 3kb.

Reaction setup

1. Prepare a PCR master mix based on following table (and briefly vortex Azura 2x 1-Step Ultra Mix):

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

2. Reverse Transcription and PCR cycling:

Cycles	Temperature & Time	Notes
1	45°C, 15 minutes	Use 50°C for high degree of secondary structure only
1	95°C, 3 minutes	Initial Denaturation, polymerase activation
30 - 40	95°C, 10 seconds 55°C to 67°C, 10 seconds 68°C, 40 seconds to 60 seconds	Denaturation Annealing (determined by user) Extension

Quality Control

Azura One-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 One-Step Ultra RT-PCR Kit is manufactured under a comprehensive quality management system, following ISO 9001:2008 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.

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