

## AzuraQuant™ cDNA Synthesis Kit

Catalog No.	Pack Size and Concentration	Components and Volume	
AZ-1995	25 Reactions, 5x	HighTherm™ RTase - 1 x 25µl	5x cDNA Buffer – 1 x 100µl
AZ-1996	100 Reactions, 5x	HighTherm™ RTase - 4 x 25µl	5x cDNA Buffer – 4 x 100µl

### Description

The AzuraQuant™ cDNA Synthesis Kit provides a rapid and sensitive method for generating high quality, qPCR-ready first-strand cDNA for use in downstream real-time PCR. The AzuraQuant™ cDNA Synthesis Kit exhibits highly reproducible, unbiased synthesis and representation of 5' and 3' mRNA ends. The kit was developed to provide consistent linearity across a wide range of input RNA, revealing accurate relative cDNA representation, regardless of gene abundance. HighTherm™ reverse transcriptase which provides exceptional thermostability is blended with a potent RNase inhibitor to ensure high integrity of Total RNA starting material. The AzuraQuant™ cDNA Synthesis Kit is supplied with a high-performance 5x cDNA buffer system including an optimized ratio of random hexamer primers and anchored oligo (dT) primers which delivers unbiased, efficient and sensitive cDNA synthesis. The AzuraQuant™ cDNA Synthesis Kit delivers both highly efficient first-strand synthesis and higher cDNA yields, leading to enhanced reproducibility and data accuracy, providing an ideal chemistry for success with limited starting material, such as tissue biopsies. The Kit is supplied in a convenient, two tube format comprised of a 5x cDNA Buffer and 20x HighTherm™ reverse transcriptase blended with RNase inhibitor.

- Complete 5' to 3' RNA sequence representation from as little as 4pg Total RNA.
- HighTherm™ reverse transcriptase coupled with an optimized 5x cDNA Buffer system generates consistent, high-yield qPCR-ready cDNA.
- Optimized for dilute and low-copy input RNA.

### Storage

The AzuraQuant™ cDNA Synthesis 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, the kit is stable for 12 months from date of receipt. The AzuraQuant™ cDNA Synthesis Kit may also be stored at 4°C for 1 month.

### Important Guidelines

**Azura 5x cDNA Buffer:** The 5x cDNA buffer contains optimal levels of dNTPs (5mM), 15mM MgCl<sub>2</sub>, enhancers, stabilizers, and a unique ratio of random hexamers and anchored oligo (dT) primers. The buffer has been designed to deliver maximum efficiency and very high-quality qPCR-ready cDNA. We do not suggest the use of additional reaction components.

**Template:** Ideally, we suggest the use of 4.0 pg to 0.5 µg Total RNA or oligo(dT) purified mRNA to maintain accurate relative cDNA representation. HighTherm™ reverse transcriptase exhibits greater activity than many competing enzymes and generates very high cDNA yields, from small amounts of RNA.

**Incubation temperature:** We recommend a temperature of 42°C for 30 minutes for the vast majority of applications (<65% GC content). Incubation temperatures of up to 55°C may be used for regions containing significant secondary structure (>65% GC content).

**qPCR reaction set-up:** The cDNA produced can be diluted 10x in PCR-grade water prior to qPCR although the optimum dilution should be determined based on target gene abundance. We recommend 2.0 µl to 5.0 µl of the cDNA solution per 20µl real-time PCR reaction. Alternatively, cDNA may be stored at 4°C for 1 week or -20°C for long term storage.

## Reaction setup

1. Prepare a master mix based on following table (and allow 5x cDNA buffer to thaw):

Component	20µl Reaction	Final Concentration/Notes
5x AzuraQuant™ cDNA Buffer	4 µl	1x
20x HighTherm RTase	1.0 µl	Add prior to RNA template
Total RNA (4pg – 0.5µg)	variable	Total RNA or mRNA
PCR-grade water	Up to 20 µl final volume	

\* For alternative total reaction volumes (eg. 25 µl), scale all components proportionally to maintain final concentrations.

2. Incubation and subsequent enzyme denaturation:

	Temperature & Time	Notes
Incubation	42°C, 30 minutes	For RNA with high degree of secondary structure, incubate at 55°C
Denaturation	85°C, 10 minutes	This will denature RTase

## Quality Control

The AzuraQuant™ cDNA Synthesis Kit is tested extensively for reproducibility, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. The AzuraQuant™ cDNA Synthesis 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](mailto:tech@azuragenomics.com) and provide reaction conditions including incubation temperature and time, and RNA concentration.

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