AzuraQuant™ II cDNA Synthesis Kit

Catalog No.	Pack Size and Concentration	Components and Volume	
AZ-2501	25 Reactions, 5x	HighTherm™ II RTase - 1 x 25 µl	5x cDNA synthesis mix – 1 x 100 µl
AZ-2504	100 Reactions, 5x	HighTherm™ II RTase - 4 x 25 µl	5x cDNA synthesis mix – 4 x 100 µl
AZ-2520	500 Reactions, 5x	HighTherm™ II RTase - 10 x 50 µl	5x cDNA synthesis mix – 10 x 200 µl

Description

The AzuraQuant™ II cDNA Synthesis Kit has been developed for fast and sensitive first-strand cDNA synthesis. The kit was developed to provide unbiased synthesis across a wide range of input RNA, up to 2 µg. The AzuraQuant™ II cDNA Synthesis Kit is supplied with a high-performance 5x cDNA synthesis mix including an optimized ratio of random hexamer primers and anchored oligo (dT) primers which delivers unbiased, efficient and sensitive cDNA synthesis. The AzuraQuant™ II cDNA Synthesis Kit delivers both highly efficient first-strand synthesis and higher cDNA yields, leading to enhanced reproducibility and data accuracy. The kit is supplied in a convenient, two tube format comprised of a 5x cDNA synthesis mix and 20x HighTherm™ II reverse transcriptase blended with RNase inhibitor.

- Complete 5' to 3' RNA sequence representation from as little as 4 pg.
- HighTherm™ II reverse transcriptase coupled with an optimized 5x cDNA synthesis mix generates consistent, high-yield qPCR-ready cDNA.
- Optimized for a wide dynamic range of input RNA.

Storage

The AzuraQuant™ II cDNA Synthesis Kit is shipped on blue ice and should be stored at –20°C upon receipt. Excessive freeze/thawing should be avoided.

Important Guidelines

5x cDNA synthesis mix: The 5x cDNA synthesis mix contains optimal levels of dNTPs, $MgCl_2$, enhancers, 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 additives.

Template: Ideally, we suggest the use of up to 1 µg Total RNA to maintain accurate relative cDNA representation, although the kit has been successfully tested with up to 2 µg.

Incubation temperature: We recommend a pre-incubation step of 25°C for 10 minutes followed by cDNA synthesis at 50°C for 10 minutes for the vast majority of applications.

qPCR reaction set-up: The cDNA produced can be diluted 5x -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 cDNA synthesis mix	4 µl	1x
20x HighTherm II RTase	1.0 µl	Add prior to RNA template
Total RNA (4 pg – 1 µg)	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
Pre-incubation	25°C, 10 minutes	Primer annealing step
Incubation	50°C, 10 minutes	cDNA synthesis step
Denaturation	85°C, 5 minutes	This will denature RTase

Quality Control

The AzuraQuant™ II cDNA Synthesis Kit is tested extensively for reproducibility, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. The AzuraQuant™ II cDNA Synthesis 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 reaction conditions including incubation temperature and time, and RNA concentration.