AzuraFlex™ cDNA Synthesis Kit

Catalog No. and Pack Size	Kit Components
AZ-1997 200 Reactions **AZ-1998 1000 Reactions**	• **High Capacity RTase 200 U/µl** • **5x RTase Buffer** • **Random hexaprimer 100 µM** • **Oligo (dT) primer, 100 µM**

Description

The AzuraFlex™ cDNA Synthesis Kit is a complete and flexible system for efficient synthesis of first strand cDNA from Total RNA or mRNA templates. The MMLV-derived reverse transcriptase (High Capacity RTase) is modified for improved sensitivity with challenging templates, and greater thermostability than conventional MMLV. The enzyme, which produces high-quality cDNA is blended with a potent RNase inhibitor for greater stability and performance with a wide range of RNA sources.

- Includes separate 100 µM solutions of random hexamer primers and oligo (dT) primers for assay flexibility.
- System generates consistent, high-yield qPCR-ready cDNA
- Optimized for a wide range of input RNA of 10 pg to 2 µg Total RNA.
- Downstream applications include real-time PCR, standard PCR, and microarrays.
- Optimized 5x RTase Buffer includes optimized levels of dNTPs and $MgCl_2$ for reduced pipetting steps.

Storage

The AzuraFlex™ 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 RTase Buffer: The 5x RTase Buffer contains optimized levels of 5 mM dNTPs, 15 mM $MgCl_2$, enhancers, and stabilizers. The buffer has been formulated 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 10 pg to 2.0 µg total RNA or 5 pg to 0.5 µg of oligo(dT) purified mRNA to maintain accurate relative cDNA representation. As concentrations of target sequences will vary, users are encouraged to perform a template titration to find the optimal concentration for a given application.

Incubation temperature: We recommend a temperature of 42°C for 30 minutes for the vast majority of applications (<65% GC content). Incubation temperatures 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 20 µl 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. Allow Kit components to thaw and mix by inversion/centrifuge. Keep tubes on ice during reaction setup. For non-biased amplification, we suggest a blend of both random hexaprimers and oligo $(dT)_{18}$. Prepare **a 10x Primer Blend** in PCR-grade water for use in the cDNA synthesis reaction according to the following:

Oligo Type	Reaction concentration	10x Primer Blend
Random hexaprimers	1.0 – 4 µM	10 - 40 µM
oligo (dT)	0.10 – 1.0 µM	1.0 – 10 µM

NOTE – For Gene specific primers, we recommend a reaction concentration in the range of 0.2 - 1 µM

2. Reaction Set-Up: Prepare a mix based on the following, adding reagents in the sequence listed in table:

Reagent	20 µl Reaction	Notes
5x RTase Buffer	4 µl	
High Capacity RTase	1.0 µl	RTase/RNase inhibitor blend
Total RNA or mRNA	x µl (10 pg – 2.0 µg RNA)	See Important Guidelines
10x Primer Blend	2 µl	See 10x Primers Mix section
PCR-Grade Water	Up to 20 µl final volume	

NOTE – For alternative reaction volumes, scale all reagents proportionally to maintain final concentrations.

3. 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

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.