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AzuraFlex™ cDNA Synthesis Kit

Catalog No. and Pack Size	Kit Components
AZ-1997 200 Reactions **AZ-1998 1000 Reactions**	• **High Capacity RTase 100u/µl** • **5x RTase Buffer** • **Random Hexaprimer 20µM** • **Oligo (dT) primer, 20µM**

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. The enzyme, which is capable of synthesizing cDNA up to 9Kb is blended with RNase inhibitor for greater stability and performance with a wide range of RNA sources.

- Includes separate solutions of random hexamer primers and oligo (dT) primers for assay flexibility.
- System generates consistent, high-yield qPCR-ready cDNA fragments up to 9Kb in length.
- Optimized for a wide range of input RNA of 10pg to 2µg Total RNA.
- Downstream applications include real-time PCR, standard PCR, and microarrays.
- Optimized 5x RTase Buffer Mix includes optimal 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. When stored as specified, the kit is stable for 12 months from date of receipt.

Important Guidelines

5x RTase Buffer: The 5x RTase Buffer contains optimal levels of dNTPs, 20mM $MgCl_2$, enhancers, and stabilizers. The buffer has been optimized 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. For input RNA in excess of 2.0 µg in a 20 µl volume, we suggest the use of 1.5x -2.0x the suggested amounts of High Capacity RTase and primers to allow for high-yield cDNA synthesis.

Incubation temperature: We recommend a temperature of 42°C for 60 minutes for the vast majority of applications (<65% GC content). Incubation temperatures of 45°C may be used for regions containing significant secondary structure (>65% GC content). If using random hexaprimers, incubate for 10 minutes at 25°C and then 60 minutes at 42°C.

qPCR reaction set-up: We recommend 2.0µl to 4.0µl of the cDNA produced 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. Allow Kit components to thaw and briefly vortex/centrifuge. Keep tubes on ice during reaction setup. Prepare **RNA/Primer Mix** - Combine the following components in a nuclease-free microtube on ice:

RNA/Primer Mix	12 µl Total Volume	Final Concentration/Notes
RNA Template	10 pg to 2.0 µg Total RNA	5 pg to 0.5 µg mRNA
Random Hexaprimer or oligo (dT)	2.0 µl	Use 3 µl for >2 µg RNA
PCR-Grade Water	Up to 12 µl final volume	

NOTE – Briefly vortex and centrifuge. Heat RNA/Primer Mix at 70°C for 2 minutes in order to relax secondary structures and place tubes on ice.

2. In a separate tube, prepare a general volume of **RTase Mix** (determined by the required number of reactions) of the following:

RTase Mix	8µl Volume Per Reaction	Notes
5x RTase Buffer	4 µl	
High Capacity RTase	1.0 µl	Use 2 µl for >2 µg RNA
PCR-Grade Water	Up to 8 µl final volume	

NOTE – For example, to prepare sufficient RTase Mix for 96 reactions, a general volume of 768 µl is required. It is important to calculate for a small amount of excess volume to account for possible pipetting losses.

3. Add **8 µl RTase Mix** to **12 µl RNA/Primer Mix** for a total volume of 20 µl per reaction, mix by pipetting gently (or briefly vortex/centrifuge). *The use of Random Hexaprimers requires a pre-incubation of 10 minutes at 25°C before proceeding to table below.*

	Temperature & Time	Notes
Incubation	42°C, 60 minutes	For RNA with high degree of secondary structure, incubate at 45°C
Denaturation	70°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.

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