DirectAmp™ One-Step RT-qPCR Mastermix

Recommended User Guide

For Research Use Only. Not for Use in Diagnostic Procedures.

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1.1 Reagents

Reagent	Size	SKU
A2K DirectAmp RT-qPCR Mastermix	1mL	MM-DA-1
	5mL	MM-DA-5
	10mL	MM-DA-10
	25mL	MM-DA-25

2.1 Overview

The A2K DirectAmp One-Step RT-qPCR Mastermix is designed for use in probe-based, real-time quantitative PCR. The A2K DirectAmp One-Step RT-qPCR Mastermix is 4X concentrated and contains a hot-start reverse transcriptase, a hot-start DNA polymerase, dNTPs, $MgCl_2$, enhancers, and stabilizers.

In addition, an enhancer solution is provided as an optional additive to neutralize PCR inhibitors including nucleases in crude samples.

2.2 Shipping & Storage

The A2K DirectAmp One-Step RT-qPCR Mastermix is shipped on dry ice. Upon receipt, store at -15 to -30°C in a constant temperature, non-frost free freezer.

3.1 Recommendations for Use

- Setup qPCR plate on ice or at 4°C.
- Minimize time between addition of template and starting the qPCR run. Depending on stability of template, reaction ready plates containing reaction-mix and **no** template may be stored at 4°C for 8 hours before the qPCR run without negatively affecting performance of the Mastermix.
- If plate is setup at room temperature then qPCR run should be initiated no more than 30 minutes after template addition.

4.1 Preparation

1. Thaw Mastermix along with other required reagents i.e. assay, template, etc.
2. Mix reagents thoroughly. Use inversion for Mastermix.
3. Centrifuge to collect solution at bottom of tubes if needed.

4.2 Reaction Mix

1. Determine the total number of reactions in your experiment, including replicates, controls, and add 1-3 additional reactions to account for pipetting errors (overage).
2. Combine all components except for the template and invert 4-6 times to mix:

A2K DirectAmp Mastermix qPCR Setup for Crude Sample		
Component	**Volume per 20µL reaction**	**Volume per 10µL reaction**
A2K DirectAmp Mastermix	5µL	2.5µL
A2K DirectAmp Enhancer	1-2µL	0.5-1µL
Assay	Varies	Varies
Template **(Do not add to reaction mix)**	Varies	Varies

Nuclease-Free Water	Varies	Varies

4.3 Prepare qPCR Reaction Plate

1. Add reaction mix to wells of qPCR plate
2. Add Crude Template to well of qPCR plate (Ensure total volume is 10 or 20µL)
3. Seal qPCR plate with optical PCR film.
4. Vortex and centrifuge plate.

4.4 Setup PCR Cycling Program

Step	Cycles	Temperature (°C)	Fast Cycling (min:sec)	Standard Cycling (min:sec)
Reverse Transcription	1	50	15:00	15:00
Polymerase activation	1	95	0:30	3:00
Denaturation	35-45	95	0:05	0:15
Annealing/extension		60	0:45	1:00

4.5 Run PCR

Place the plate in the RT-PCR instrument and start the cycling program.