

## I. Description

Prelude One-Step PreAmp Master Mix is a 2X RT-PCR mix optimized for unbiased co-amplification of multiple targets (>100) in a single tube starting from low amounts of input RNA (down to 100 pg mRNA or 10 ng total RNA). Cat. Nos. 638554 and 638553 provide enough reagent for 10 and 40 reactions, respectively.

This premix works with intact RNA as well as degraded RNA extracted from FFPE samples. Amplified products can be used for downstream qPCR, genotyping, or targeted enrichment analysis. The formulation is compatible with both intercalating dye- and probe-based gene expression detection methods.

## II. Preamplification of RNA Targets

### A. Assay Primer Pooling

Combine assay primers to achieve a concentration of 25–50 nM for each primer. (See Section III.1 for more information.) Use low EDTA (0.1 mM) TE buffer as the diluent.

### B. Reaction Setup

Combine the following components. A typical reaction volume of 50 µl is illustrated below:

25 µl	Prelude One-Step PreAmp Master Mix
5 µl	Primer pool (from Section II.A; see Section III.1)
1 µl	Input RNA (e.g., 10 ng/µl total RNA)
19 µl	PCR-grade water
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50 µl	Total volume

### C. Recommended Cycling Conditions

Cycle the reaction as needed based on the sample input amount (additional guidelines in Section III.2):

42°C	10 min	} 10–14 cycles
95°C	2 min	
95°C	10 sec	
60°C	4 min	
4°C	Hold	

## III. General Considerations

1. Preconfigured primer sets or custom-designed assay primer sets can be used with Prelude One-Step PreAmp Master Mix. For optimal performance, primers should be pooled to achieve a final, target concentration in the reaction of 2.5–5 nM for each primer.
2. Depending on the sample input amount, the PCR program should be cycled 10–14 times. Cycles can be extended up to 18 cycles, although minor loss of amplification uniformity may occur.

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