I. Description

Prelude PreAmp Master Mix is a 2X PCR mix optimized for unbiased co-amplification of multiple (>100) targets in a single tube starting from low amounts of input sample (10 ng–100 pg cDNA). It contains an antibody-based hot start function for increased specificity, enabling room temperature reaction assembly. This premix works with intact gDNA and cDNA, as well as degraded DNA extracted from FFPE samples. Downstream applications include gene expression profiling and targeted resequencing; the formulation is compatible with both intercalating dye- and probe-based gene expression detection methods.

II. Preamplification of cDNA or gDNA targets

A. Pooling the assays

Combine equal volumes of each assay and dilute with low EDTA (0.1 mM) TE buffer to achieve a target assay concentration of 500 nM.

B. Reaction setup

A typical reaction volume of 50 µl is illustrated below; lower volumes can also be used:

25 µl	Prelude PreAmp Master Mix
5 µl	Primer pool (500 nM stock from Section II.A)
1 µl	Input cDNA (100 pg/µl)
19 µl	PCR-grade water
50 µl	Total volume

C. Recommended cycling conditions

Cycle the reaction as needed for the sample input amount (10–14 cycles):

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

III. General Considerations

1. For optimal performance, primers are pooled to target a final concentration of 25–50 nM in the amplification reaction; these can be preconfigured primer/probe sets or custom-designed assay primer sets. Lower concentrations (0.1 to 1 nM) have been tested and also work well.

2. Depending on the sample input amount, the PCR program is cycled 10–14 times. The number of cycles can be extended further (up to 28 cycles), although minor loss of amplification uniformity may occur as primer concentrations become limiting (above 18 cycles).

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This document has been reviewed and approved by the Quality Department.