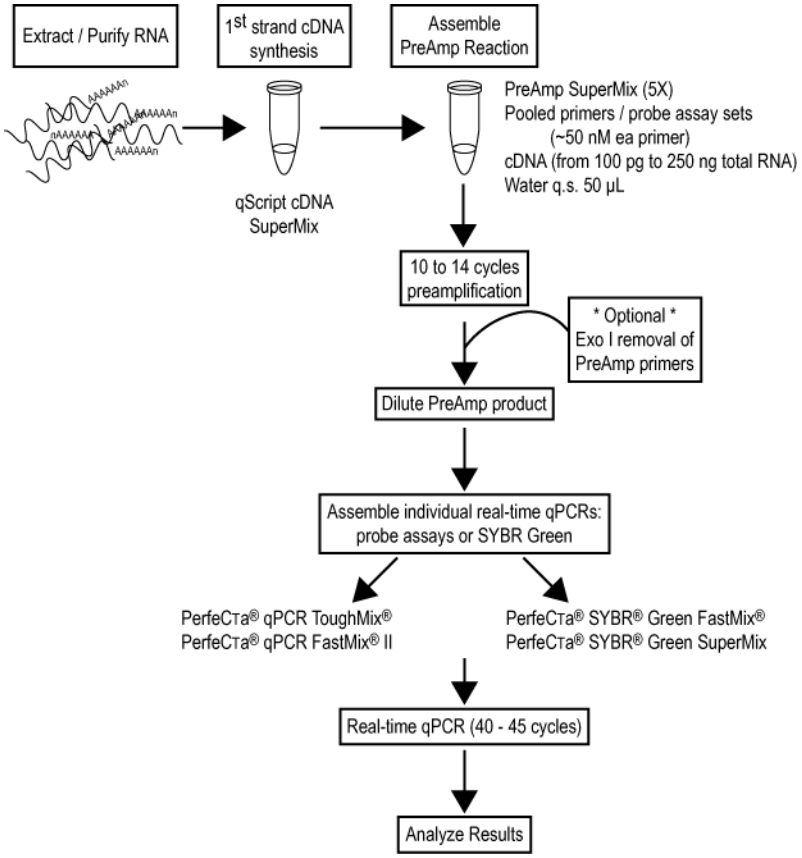
PerfeCTa® PreAmp SuperMix

Cat. No. 95146-005 Size: 5x 50-µL reactions Store at -20ºC
95146-040 40 x 50-µL reactions

Description

PerfeCTa PreAmp SuperMix is a 5X concentrated, ready-to-use reaction cocktail for unbiased, selected enrichment of target sequences from limiting amounts of starting material for downstream gene expression profiling or targeted re-sequencing. It contains all components, except primers and templates. The 5X concentrated SuperMix allows addition of higher template volumes when working with low concentration samples, and/or reduced reaction volumes. Inclusion of an inert light blue tracer dye helps visualize small reaction volumes and ensure accurate pipetting. PerfeCta PreAmp SuperMix delivers unbiased pre-amplification of up to 100 target sequences from as little as 100 pg of total RNA. It is compatible with both TaqMan® 5'-nuclease probes or ds-DNA binding dye (i.e. SYBR® Green I) qPCR detection chemistries.

A key component of PerfeCTa PreAmp SuperMix is an ultra-pure, highly processive, thermostable DNA polymerase that is combined with high avidity monoclonal antibodies. This proprietary polymerase mix is resistant to PCR inhibitors and provides an extremely stringent automatic hot-start allowing reaction assembly, and temporary storage, at room temperature prior to pre-amplification.



PreAmp Process Flow:

1. Prepare RNA
2. Reverse transcribe RNA
3. Pool assay primers and dilute
4. Perform pre-amplification reaction
5. Dilute PreAmp reaction product
6. Perform individual qPCRs for each pre-amplified gene of interest (GOI).

Components

PerfeCTa PreAmp SuperMix	5X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), hot-start DNA polymerase, AccuVue™ blue tracer dye, and stabilizers.

Storage and Stability

PerfeCTa PreAmp SuperMix is stable for 1 year when stored in a constant temperature freezer at -20ºC, protected from light. For convenience, it may be stored unfrozen at +2 to +8ºC for up to 6 months. Repeated freezing and thawing of the supermix will not affect product performance.

Pooling of Assay Primers

For TaqMan® assays:

- Combine equal volumes of each 20X TaqMan Gene Expression Assay (up to 100 assays) and dilute with $T_{10}E_{0.1}$ buffer to achieve a final concentration of 0.2X for each assay (180 nM each primer, 50 nM probe).
- For example, to pool 50 assays combine 10 µL of each 20X assay and add 500 µL of $T_{10}E_{0.1}$ buffer making a total of 1 mL at 0.2X of each assay.
- $T_{10}E_{0.1}$ buffer = 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA

For SYBR® Green assays,

- Combine equal volumes of all forward and reverse primers and dilute with $T_{10}E_{0.1}$ buffer to achieve a final concentration of 0.5 µM of each assay primer.
- For example, to pool 50 assays (50 forward and 50 reverse primers at 100 µM each) combine 5 uL of each forward and reverse assay primer and add 500 µL of $T_{10}E_{0.1}$ buffer making a total of 1 mL at 0.5 µM each primer.
- $T_{10}E_{0.1}$ buffer = 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA

Pre-amplification Reaction Setup for TaqMan Assays

Component	Final Concentration	Volume (µL)
PerfeCTa PreAmp SuperMix (5X)	1X	10.0
TaqMan Assay Pool (0.2X each assay)	0.05X each (45 nM each primer, 12.5 nM probe)	12.5
0.1 – 250 ng cDNA (total RNA equivalent)	Variable	Variable
Nuclease-free water		Variable
Total		50.0

Pre-amplification Reaction Setup for SYBR Green Assays

Component	Final Concentration	Volume (µL)
PerfeCTa PreAmp SuperMix (5X)	1X	10.0
Assay Primer Pool (0.5 µM each assay)	50 nM each primer	5.0
0.1 – 250 ng cDNA (total RNA equivalent)	Variable	Variable
Nuclease-free water		Variable
Total		50.0

Note: For smaller or larger pre-amplification reaction volumes, scale all components proportionally. Pre-amplification can be carried out in a little as 5 to 10 µL depending on your application requirements. For best results only use assays with high PCR efficiency. Primer designs should follow real-time PCR design guidelines with melting temperatures (Tm) between 58ºC and 62ºC.

- Seal tubes, or PCR reaction plate, and mix by gently vortexing.
- Briefly centrifuge to collect reaction contents in the bottom of each tube or well.
- Run the PreAmp reaction.

Pre-amplification Cycling Protocol

Incubate assembled reactions in a conventional thermal cycler as follows:

Initial denaturation:	95ºC, 2 min
PreAmp cycling (10 *or* 14 cycles):	95ºC, 10 s
	60ºC, 3 min
Hold	4ºC

Optimal number of pre-amplification cycles is dependent on the amount of starting template, relative abundance of your specific GOI and the number of pre-amplified GOI. When using highly efficient assays, 10 cycles should enrich 1,024-fold; 14 cycles should enrich 16, 384-fold.

Dilution of PreAmp Product

Transfer PreAmp reactions to ice.

Dilute PreAmp reaction products according to the number of pre-amplification cycles used – or based on the requirements of your specific experiment designs. As a general guideline:

- Dilute 10-cycle PreAmp reactions 1:5 using cold $T_{10}E_{0.1}$ buffer (pH 8.0).
- Dilute 14-cycle PreAmp reactions 1:20.

qPCR Amplification

Amplify 2 – 10 µL of diluted PreAmp product using your preferred detection chemistry according to the instructions provided with your real-time PCR reagent and choice of real-time PCR instrument. Optimal input amount may vary depending on qPCR reaction volume and PreAmp product dilution factor.

For probe-based qPCR we recommend PerfeCTa qPCR FastMix II. Use TaqMan Gene Expression Assays at a final concentration of 0.5X (450 nM each primer, 125 nM probe). We suggest a cycling protocol of:

Initial denaturation:	95ºC, 1 min
qPCR cycling (40-45 cycles):	95ºC, 5 s
	60ºC, 30 s

For SYBR Green qPCR we recommend PerfeCTa SYBR Green FastMix or PerfeCta SYBR Green SuperMix. Use 200 nM of each gene-specific primer and a cycling protocol of:

Initial denaturation:	95ºC, 1 min
qPCR cycling (40-45 cycles):	95ºC, 5 s
	60ºC, 30 s

Quality Control

Kit components are free of contaminating DNase and RNase. PerfeCTa PreAmp SuperMix is functionally tested in 96-plex PreAmp reactions using cDNA prepared from 10 ng of a universal reference total RNA. Pre-amplifications are performed in triplicate for both 10 and 14 cycles. Each of the 96 individual assays are then assayed by SYBR Green qPCR using input amounts of pre-amplified cDNA normalized to 4 ng of the original cDNA. Cq values for each assay are compared to control qPCRs from 4 ng of the original cDNA. >90% of assays are within +/- 1.5 ΔΔCq. R^2 value of Cq values between cDNA and pre-amplified cDNA should be >0.97 for at least 95% of the assays. R^2 value of Cq values between cDNA pre-amplified for 10 cycles and 14 cycles should be 0.99 for >95% of the assays.

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