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USER MANUAL PCR002

PCR 20/20 Plus

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Enhanced DNA polymerase specificity throughout your PCR reaction with hot-start enzyme control combined with mis-priming suppression

INCLUDED

- PCR 20/20 lyophilized powder; 500 or 2500 units
- Focus; 500 or 2500 units
- Resuspension buffer

PRODUCT DESCRIPTION

PCR 20/20 Plus is a kit designed to increase the reproducibility of both real-time and end-point PCR analysis and enhance enzyme specificity. The first reagent, PCR 20/20, is a reversible hot start and cold stop reagent that prevents non-specific enzymatic activity. The second component, Focus, is a double-stranded, chemically modified nucleic acid that suppresses mis-priming during the annealing and extension steps of PCR.

PRODUCT PREPARATION

Kit components (PCR 20/20 and Focus) are shipped lyophilized as dry powder. To prepare the stock solution of 5 U/µl each, add resuspension buffer (molecular grade 10 mM Tris-HCL pH 8.0) to each tube of dry reagent. Vortex 1-2 minutes, then centrifuge briefly. Allow tubes to sit at room temperature for 15 minutes, mixing occasionally to ensure reagent is completely dissolved. Centrifuge before using. See the table below for reconstitution protocol.

Product Name	Pack Size	Buffer Amount
PCR 20/20 PCR001	500 Units	100 µl Resuspension buffer
	2500 Units	500 µl Resuspension buffer
Focus PCR004	500 Units	100 µl Resuspension buffer
	2500 Units	500 µl Resuspension buffer

Note: One unit each of PCR 20/20 and Focus is defined as the amount required for maximum improvements in specificity, yield, and reproducibility in amplification reactions containing 1 unit of Taq DNA polymerase in a volume of 25 µl.

PCR 20/20 PLUS PROTOCOL

To use: Add an equal number of units of PCR 20/20, Focus and Taq DNA polymerase to the PCR master mix. PCR annealing temperature should be 60°C or above to ensure full polymerase activity. PCR 20/20 combined with Focus works synergistically to improve PCR performance before, during, and after amplification.

For a 25 µl PCR reaction set up:

Reagent	Final Concentration	Volume
10X PCR Buffer	1X	2.5 µl
2 µM primers	0.2 µM	2.5 µl
5 U/µl Taq	0.05 U/µl	0.25 µl
5 U/µl PCR 20/20	0.05 U/µl	0.25 µl
5 U/µl Focus	0.05 U/µl	0.25 µl
Template		X µl
Water		fill to final volume of 25 µl

CANOPY BIOSCIENCES

Mix the Taq DNA polymerase, PCR 20/20, and Focus together before adding the remaining reaction mixture components. It is critical for PCR 20/20 and Focus to interact with the Taq DNA polymerase enzyme **before** it is mixed with primers and/or template in order for it to suppress polymerase activity appropriately. Add the remaining reagents and proceed with PCR thermocycler protocol.

PCR 20/20 was evaluated for reaction volumes of 10-25 µl. For volumes outside this range, optimization may be needed.

It may be necessary to titrate the amount of Focus. Test the reagent in the range of 0.5 to 2.0 units per unit of Taq DNA polymerase to obtain improved product specicity without reducing amplification efficiency.

Recommended storage:

Store stock solutions of PCR 20/20 and Focus at 4°C or -20°C in the dark or light-protected vials. If frozen, divide stock into small volume aliquotes to avoid freezing and thawing more than 5 times.