



USER MANUAL PCR001

PCR 20/20

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PCR 20/20

Innovative hot start and cold stop reagent for enhanced Taq DNA polymerase specificity and product yield

INCLUDED

Lyophilized powder
500 or 2500 units

PRODUCT DESCRIPTION

PCR 20/20 is a reversible hot start reagent that is compatible with all taq DNA polymerases. It acts directly on the polymerase to prevent non-specific enzymatic activity below 50°C. Polymerase activity is fully restored at 60°C, but is suppressed again by PCR 20/20 upon reaction cooling.

PRODUCT PREPARATION

PCR 20/20 is shipped as a lyophilized dry powder in 500 or 2500 units. To prepare a stock reagent of 5 U/ μ l add: 100 μ l of molecular grade 10 mM Tris-CL pH 8.3 to 500 units of dry reagent (use 500 μ l 10 mM Tris-CL pH 8.3 for 2500 unit pack size), vortex 1-2 minutes, then centrifuge briefly. Allow tube to sit at room temperature for 15 minutes, mixing occasionally to ensure reagent is completely dissolved. Centrifuge before using.

Note: One unit of PCR 20/20 is defined as the amount required for maximum hot start activity in amplification reactions containing 1 unit of Taq DNA polymerase in a volume of 25 μ l. PCR 20/20 does not contain magnesium, dNTPs, or other PCR buffer components.

PCR 20/20 PROTOCOL

To use: Add an equal number of units of PCR 20/20 and Taq DNA polymerase to the PCR master mix. PCR annealing temperature should be 60°C or above to ensure full polymerase activity.

For a 25 μ l PCR reaction set up:

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

Mix the first 2 components, Taq DNA polymerase and PCR 20/20, together before adding the remaining reaction mixture components. It is critical for PCR 20/20 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.

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



Recommended storage:

Store PCR 20/20 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.