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PCR 20/20 RT

Improved RT-PCR sensitivity, specificity and product yield

INCLUDED

Lyophilized powder
250 or 1250 units

www.canopybiosciences.com

USER MANUAL PCR003

PCR 20/20 RT

PRODUCT DESCRIPTION

PCR 20/20 RT is a simple-to-use reagent for one-step or two-step reverse transcription PCR (RT-PCR). PCR 20/20 RT interacts with reverse transcriptase at low temperatures to reduce priming errors that lead to non-specific products. By eliminating these errors, PCR 20/20 RT significantly increases the yield of the desired specific products and increases assay sensitivity.

PRODUCT PREPARATION

PCR 20/20 RT is shipped as a lyophilized dry powder in 250 or 1250 units. To prepare a stock reagent of 5 U/µl add: 50 µl of molecular grade 10 mM Tris-CL pH 8.3 to 250 units of dry reagent (use 250 µl 10 mM Tris-CL pH 8.3 for 1250 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 RT is defined as the amount required for optimal performance in RT-PCR samples containing 50 units of reverse transcriptase and one unit of hot start Taq DNA polymerase in a volume of 20 µl and a reaction temperature of 50°C. PCR 20/20 RT does not contain magnesium, dNTPs, or other PCR buffer components.

PCR 20/20 RT PROTOCOL FOR TWO-STEP RT-PCR

For a 20 µl cDNA synthesis reaction:

Reagent	Final Concentration	Volume
RNase-free water		12.15 µl
5X RT buffer	1X	4 µl
10 mM dNTPs	0.4 mM	0.8 µl
5 U/µl PCR 20/20 RT	0.05 U/µl	0.2 µl
200 U/µl reverse transcriptase	2.5 U/µl	0.25 µl
10 µM primers	0.3 µM	0.6 µl
RNA template		2 µl

1. Mix the first 5 components before adding primers and RNA template. Primers and RNA template can be prepared in a separate mixture with the RT buffer and incubated at an appropriate annealing temperature, if desired, before mixing with other components.
2. Incubate cDNA synthesis mixture at 50°C or higher for 15-30 minutes OR 45°C for 30-60 minutes, then at 95°C for 2 minutes.
3. Dilute aliquotes of the cDNA sample 4- to 10-fold with PCR reagent mixture and perform thermal cycling as usual.

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PCR 20/20 RT PROTOCOL FOR ONE-STEP RT-PCR

For a 20 µl cDNA synthesis reaction:

Reagent	Final Concentration	Volume
RNase-free water		11.95 µl (adjust as needed for a final reaction volume of 20 µl)
5X RT-PCR buffer	1X	4 µl
10 mM dNTPs	0.4 µM	0.8 µl
5 U/µl PCR 20/20 RT	0.05 U/µl	0.2 µl
200 U/µl reverse transcriptase	2.5 U/µl	0.25 µl
5 U/µl hot start Taq	0.05 U/µl	0.2 µl
10 µM primers	0.3 µM	0.6 µl
RNA Template		0. 5 to 5 µl

1. It may be necessary to test the range of PCR 20/20 RT concentrations (ie 0.5 to 4 units) for RT-PCR kits containing unknown units of reverse transcriptase.
2. Mix the first 6 components before adding primers and RNA template. Primers and RNA template may be prepared in a separate mixture with the RT-PCR buffer and incubated at an appropriate annealing temperature, if desired, before mixing with other components.
3. Incubate cDNA synthesis mixture at 50°C or higher for 15-30 minutes OR 45°C for 30-60 minutes, then at 95°C for 2 minutes. Then use the thermal cycling protocol appropriate for the primer and buffer conditions contained.

Recommended storage:

Store stock solution of PCR 20/20 RT 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.

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