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New Hot Rescue DNA Polymerase

1 x 250U

MBR0001D

Intended use

DNA Amplification

Product description

New Hot-rescue DNA Polymerase is a recombinant Taq DNA Polymerase complexed with a inhibitor of the polymerase activity. Due to specific binding of the inhibitor, *New* Hot Rescue DNA Polymerase is provided in an inactive form, thereby preventing nonspecific amplification. *New* Hot Rescue DNA Polymerase is activated by the initial denaturation step of PCR: 1 minute at 95°C is enough to fully release the polymerase activity.

Components

1x MBR0001A - Hot Rescue DNA Polymerase (250 U)
1x MBR0004A - dNTP mix – 2.5mM each (800 µl)
1x MBR0040 - 10X PCR Buffer V (containing 20mM Mg^{2+}, 1 ml)

Storage buffer

20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Tween20, 0,5% Nonidet P-40.

Reaction buffer (10X)

250 mM TAPS (pH9.3), 500mM KCl, 20mM $MgCl_2$, 1mM DTT.

Storage

–20°C

General protocol

1. Prepare PCR Master Mix adding the reagents as indicated below:

Component	Volume per reaction (µl)	Final Concentration
Water	-	-
10X PCR Buffer V	5	1x
dNTP mix 2.5mM each	4	200 µM each
User-provided primer 1	1-5	0.2-1 µM
User-provided primer 2	1-5	0.2-1 µM
User-provided template	-	< 1µg/reaction
Hot Rescue DNA Polymerase (5U/µl)	0.25	1.25 Units/reaction
Total Volume	50 µl	

- Any combination of water and template can be used as long as the total volume of Master Mix, sample and primers equals 50µl

- Use experimental template preferably >10^4 copies of template but < 1µg DNA/reaction.

2. Run the appropriate PCR program as indicated below.

STEP	TIME	TEMPERATURE
Initial activation step	1 min	95°C
Denaturation	15 s	95°C
Annealing	15-30 s	X°C (Approximatively 5 to 8°C below Tm of primers)
Extension	15-45 s	60-72°C
Cycle number	30-50	-

Cycle number depends on the amount of template DNA.
Set up a single step (annealing – extension) at ≥ 60°C for 30-60 s when the Tm of primers is similar to the extension temperature.