

RighTaq® is a recombinant Taq DNA polymerase purified from *E.coli* PVG-AI. This enzyme is complexed with a monoclonal antibody blocking the polymerase activity at ambient temperature. Heat activation of **RighTaq®** occurs during the first denaturation step. An active complex of **RighTaq®** dissociates automatically over 70°C, allowing activation of DNA polymerase. No additional heating step for polymerase activation is required. This procedure gives an automatic hot start for Taq DNA polymerase in PCR⁽¹⁾ increasing sensitivity, specificity and yield.

RighTaq® can eliminate amplification artifacts such as primer-dimer formation and mispriming. The lack of activity at room temperature allows to prepare the reactions avoiding to keep the PCR mixes in wet ice. **RighTaq®** helps to reduce PCR optimization, handling of reaction components, and contamination risk, improving PCR results. **RighTaq®** is supplied at the concentration of 5 units per microlitre as **EuroTaq**, our standard DNA Polymerase (Cat.# EME010001). No modifications of PCR reactions or protocols are needed.

RighTaq® is also optimized for real time PCR protocols.

Unit definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 74°C under the following reaction conditions: 25mM TAPS tris-(hydroxymethyl)-methyl-amino-propanesulfonic acid, sodium salt pH 9.3; 50mM KCl; 2mM MgCl₂; 1mM β-mercaptoethanol; 200µM of each dNTP; 100µM dCTP (a mix of unlabeled and α-³²P labelled); 12.5µg activated salmon sperm DNA, in a final volume of 50µl.

Storage buffer

20mM Tris-HCl (pH 8.0); 100mM KCL; 0.1mM EDTA; 1mM DTT; 50% glycerol; 0.5% Nonidet P-40; 0.5% Tween-20.

10X Reaction buffer

166mM (NH₄)₂SO₄; 670mM Tris-HCl (pH 8.8 at 25°C); 0.1% Tween-20.

Application protocol

The optimal conditions (incubation times and temperatures, concentrations of **RighTaq®**, template DNA, primers, Mg²⁺, depend on the system used and have to be determined individually. Especially the Mg²⁺ concentration and the amount of enzyme used per assay should be titrated for optimal efficiency of DNA synthesis. Optimal Mg²⁺ concentrations are in the range of 1-5mM. Optimal enzyme concentrations range from 0.5-2.5 units/assay. Typically 2.5 units enzyme per reaction are used.

PCR reaction conditions

Briefly centrifuge all reagents before beginning the procedure.

1. Prepare an amplification mixture by adding reagents to a sterile microfuge tube in the following order

Reagent	Volume	Final Concentration
sterile redistilled H ₂ O	variable	-
10X Reaction buffer	5µl	1x
MgCl ₂	1-5µl	1-5mM
10mM dATP	1µl	0.2mM
10mM dCTP	1µl	0.2mM
10mM dGTP	1µl	0.2mM
10mM dTTP	1µl	0.2mM
primer 1	variable	0.1 – 1.0µM
primer 2	variable	0.1 – 1.0µM
RighTaq®	0.1 – 2.5units	0.2 - 2.5 units/50µl
DNA template	variable	<200ng/50µl
final volume	50µl	

2. Gently vortex the mixture and then centrifuge briefly to collect the sample at the bottom of the tube.

3. Amplification parameters depend greatly on the template primers and amplification apparatus used. Typically, the DNA should be amplified through 25-35 cycles of denaturation, annealing and polymerization.

Cat#	Format	Description	Volume
EME013500	2x250units	RighTaq® - 5units/µl	2 x 50µl
		10X Reaction buffer w/o Mg ⁺⁺	1 x 1.5ml
		MgCl ₂ 50mM	1 x 1.5ml

References

1. Sharkey, D.J., et al. (1994) Antibodies as thermolabile switches: high temperature triggering for the polymerase chain reaction. *Biotechnology (NY)*. 12(5):506-9.