

Euro Taq

Thermostable DNA polymerase



| Cat# | Format | Description | Volume |
|-----------|--------------|--|---------|
| EME010001 | 1x1,000units | Euro Taq - 5units/μl | 1x200μl |
| | | 10X Reaction buffer w/o Mg ⁺⁺ | 2x1.5ml |
| | | MgCl ₂ 50mM | 2x1.5ml |

STORAGE AND STABILITY

Three years at -20°C.

Euro Taq is a thermostable DNA polymerase purified from E.coli PVG-A1 recombinant strain expressing Thermus aquaticus YT1 DNA polymerase gene.

UNIT DEFINITION

One unit is defined as the amount of enzyme that incorporate 10nmoles of dNTPs into acid-insoluble form in 30 minutes at 74°C under the assay conditions, 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; 0.1mM EDTA; 1mM DTT; 50% glycerol, 0.5% Nonidet P-40, 0.5% Tween 20.

REACTION BUFFER

160mM (NH₄)₂SO₄; 670mM Tris ·HCl (pH 8.8); 0.1% Tween-20.

MAGNESIUM SOLUTION

50mM MgCl₂.

CONCENTRATION

5 units/μl.

The excellent features of this enzyme, make amplification and RT-amplification easier to set up, giving high specificity and providing a better yield.

The table below shows all the advantages of Euro Taq:

| Features | Benefits |
|-----------------------------|---|
| Multiplex PCR | Allows to set up multiplex amplifications in the shortest time |
| Long distance amplification | Allows to amplify DNA sequences up to 10kb |
| High activity level | Increased amplification yield |
| Very robust enzyme | Tolerance of different working conditions |
| High thermostability | Aggressive denaturation and stringent annealing:excellent on G-C rich templates |
| High purity | Protease free, no enzyme degradation |

APPLICATION PROTOCOL

The optimal conditions (incubation times and temperatures, concentrations of **Euro Taq**, template DNA, primers, Mg^{2+} , depend on the system used and have to be determined individually. Especially the Mg^{2+} concentration and the amount of enzyme used per assay should be titrated for optimal efficiency of DNA synthesis. Optimal Mg^{2+} 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

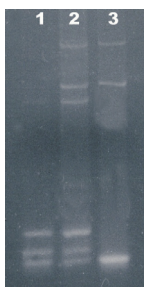
Briefly centrifuge all reagents before beginning the procedure.

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

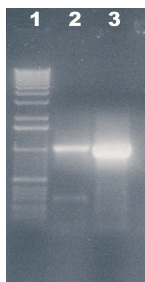
| Reagent | Volume | Final Concentration |
|--------------------------------------|----------------|----------------------|
| sterile redistilled H ₂ O | variable | - |
| 10X Reaction buffer | 5µl | 1x |
| MgCl ₂ | 1-5µl | 1-5mM |
| 10mM dATP | 1µl | 0.2mM |
| 10mM dCTP | 1µl | 0.2mM |
| 10mM dGTP | 1µl | 0.2mM |
| 10mM dTTP | 1µl | 0.2mM |
| primer 1 | variable | 0.1 – 1.0µM |
| primer 2 | variable | 0.1 – 1.0µM |
| EuroTaq | 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. For general information on amplification conditions, see (1). Typically, the DNA should be amplified through 25-35 cycles of denaturation, annealing and polymerization.



Multiplex amplifications experiments were done using **Euro Taq** (lane 1), Competitor 1 hot start Taq (lane 2) and Competitor 1 Taq (Lane 3). The expected pattern had three bands respectively at 233, 207 and 180bp. Using a not-hot start Taq we could see just the band of 180bp (lane 3), with a hot start Taq we observed the expected pattern, but non specific bands were also present (lane 2). Using **Euro Taq** we saw the three expected bands without non specific bands.



Experiments of genomic amplification were done in duplicate using **Euro Taq** and Competitor 1 Taq. The amplification program was prepared with 35 cycles and after amplification an aliquot of both the reactions were loaded onto an agarose gel and electrophoresis was performed. Lane 1 and lane 2 show the results: densitometry analysis was possible to verify that amplification done with EuroTaq (lane 3) was ten times higher than using Competitor 1 Taq (lane 2)

References

1. Erlich, H.A. (1989) PCR technology: Principles and application for DNA amplification, Stockton Press, New York.

FOR RESEARCH USE ONLY

BioCat

BioCat GmbH
Im Neuenheimer Feld 584
D-69120 Heidelberg

Tel.: +49 (0) 6221-7141516
Fax: +49 (0) 6221-7141529
E-Mail: info@biocat.com

www.biocat.com