

e2TAK DNA Polymerase (Sample)

Code No. RF001Q

Size : 40 react.

Shipping at -20°C
Stored at -20°C

Supplied Reagents :

5 × e2TAK Buffer (Mg²⁺ plus)
dNTP Mixture (2.5 mM each)

Lot No.

Volume : μ l

Expiry Date :

Description : e2TAK is a versatile DNA Polymerase optimized for PCR amplification of DNA. e2TAK has higher amplification efficiency than *Taq* DNA polymerase, and contains a 3' - 5' exonuclease activity. It is suitable for a variety of standard PCR applications.

| | | |
|--------------|--|--------|
| Components : | e2TAK DNA Polymerase | 20 μl |
| | 5 × PCR T Buffer (Mg ²⁺ plus) | 400 μl |
| | dNTP Mixture (2.5 mM each) | 180 μl |

Storage buffer : 50 mM Tris-HCl (pH 8.2 at 4°C)
100 mM NaCl
0.1 mM EDTA
1 mM DTT
50% Glycerol

Purity : Nicking, endonuclease, and exonuclease activities were not detected using supercoiled pBR322 DNA, λ DNA, or λ-*Hind* III digest DNA as substrates.

Application : For DNA amplification via the Polymerase Chain Reaction(PCR)

PCR products : The majority of the PCR products obtained using e2TAK DNA Polymerase will possess blunt ends. Thus, e2TAK products may be cloned directly into blunt-ended vectors. (If necessary, phosphorylate the PCR products before cloning.)

PCR test : Good performance of DNA amplification by PCR was confirmed by using λ DNA as the template (amplified fragment : 10 kbp). Good performance of DNA amplification of a single copy gene by PCR was also confirmed by using human genome DNA as the template (amplified fragment : approx. 4 kbp).

Supplied 5X e2TAK Buffer (Mg²⁺ plus)

| | |
|-------------------------------------|------------------|
| Size | : 400 μ l / vial |
| Mg ²⁺ concentration (5X) | : 5 mM |
| Storage | : - 20 °C |

Supplied dNTP Mixture

Mixture of dNTP, ready for use in PCR reactions without dilution.

| | |
|---------------|-------------------------------------|
| Size | : 180 μ l / vial |
| Concentration | : 2.5 mM of each dNTP |
| pH | : pH 7 ~ 9 |
| Form | : Dissolved in water (sodium salts) |
| Purity | : ≥ 98 % for each dNTP |
| Storage | : - 20 °C |

Protocol :

• **Reaction mixture preparation:**

The reaction mixture should be prepared on ice, and all necessary reagents should be stored on ice during reaction assembly. Preparing the reaction on ice decreases non-specific amplification due to primer misannealing.

Add the reagents to the reaction tube in the following order:

Sterilized distilled water, 5 × e2TAK Buffer, dNTP Mixture, e2TAK DNA Polymerase, Template DNA, Primer 1, Primer 2.

Mix the final reaction mixture by pipetting. TaKaRa recommends starting the reaction as soon as possible after reaction assembly.

• **General composition of PCR reaction mixture (50 μl)**

| | |
|----------------------------|----------------------------|
| e2TAK DNA Polymerase | 0.5 μl |
| 5 × e2TAK Buffer | 10 μl |
| dNTP Mixture (2.5 mM each) | 4 μl |
| Template DNA | < 100 ng |
| Primer 1 | 0.2 ~ 0.3 μM (final conc.) |
| Primer 2 | 0.2 ~ 0.3 μM (final conc.) |
| Sterilized distilled water | up to 50 μl |

*Recommended template amount

| | |
|----------------------------|--------------------------|
| Human genomic DNA | 5 ng ~ 100 ng (< 100 ng) |
| <i>E. coli</i> genomic DNA | 100 pg ~ 100 ng |
| λ DNA | 10 pg ~ 10 ng |
| Plasmid DNA | 100 pg ~ 10 ng |

• **PCR condition (example)**

Important Note: TaKaRa strongly recommends use of short (5 - 15 sec.) annealing times in e2TAK PCR amplifications. Longer annealing times may increase the likelihood of product smears upon gel electrophoresis.

| | |
|--------------------------|-------------|
| 98°C 10 sec. | } 30 cycles |
| 55°C 5 sec. (or 15 sec.) | |
| 72°C 1 min./kb | |
| or | } 30 cycles |
| 98°C 10 sec. | |
| 68°C 1 min./kb | |

Note:

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