*e*2TAK™ DNA Polymerase FAQ

What are the recommended annealing condtions for *e*2TAK™ DNA polymerase?

Since *e*2TAK™ DNA polymerase offers very high priming efficiency, set the annealing time at 5 sec. or 15 sec. Longer annealing times can cause smearing.

Refer to the following:

Annealing temperature:

Initially, use 5 sec at 55°C.

Annealing time:

When T_m value* is ⩾ 55°C: 5 sec.

When T_m value* is < 55°C: 15 sec.

*T_m value is calculated with the following:

Calculation formula of T_m value:

- T_m (°C) = 2 (NA + NT) + 4 (NC + NG) -5

  T_m should be calculated with above method only for a primer of less than 25 bases. When the primer is longer than 25 bases, the annealing time should be set at 5 sec.

Does Takara recommend a 3 Step PCR or a 2 Step PCR?

A 3 step PCR protocol is generally recommended.

When is a 2 Step PCR protocol recommended for *e*2TAK™ ?

Better results might be obtained with a 2 step PCR when the size of the primer is long and/or has a high GC content.

When should the longer annealing time be used?

When the size of primer is short <25 mer and/or high AT content, the 15 second annealing time might give better results.

What is the recommended template amount?

Please refer to the following:

Human genomic DNA	5 ng -100 ng(<100 ng)
E.coli genomic DNA	100 pg - 100 ng
λ DNA	10 pg - 10 ng
Plasmid	100 pg - 1 ng

Are the PCR products produced sticky or blunt-ended?

The PCR products obtained using *e*2TAK™ will possess **blunt-ends**. Thus, obtained PCR products can be directly cloned into blunt-end vectors. However, direct TA cloning is not possible.

Can *e*2TAK™ be used for colony PCR?

We do not recommend this product for colony PCR. However, a satisfactory result may be obtained by diluting the heat extracted sample.

How can I improve my cDNA template results?

When insufficient amplification product is obtained using a cDNA template, results may be improved by decreasing the amount of the template or lengthening the extension time.

What is the composition of the 5X *e*2TAK™ Buffer?

The composition of the 5X *e*2TAK™ Buffer is proprietary.

Can the denaturing temperature be set at 98°C?

98°C denaturing time is not required for this enzyme. We recommend 94°C for 30 sec. for the denaturation step. *e*2TAK™ possesses high priming efficiency, therefore a short annealing time (5 or 15 seconds) will allow high specificity amplification. If a long annealing time is tried, i.e. 30 sec, the PCR products will smear.

What is the fidelity of *e*2TAK™ ?

The fidelity of this enzyme has not been determined.

What is the half life of *e*2TAK™?

The half life of this enzyme in the PCR reaction mixture (-template, -primers) at 98°C is about 4 hours.

Can *e*2TAK™ be used with common PCR additives, such as DMSO, glycerol, BSA, or betaine?

BSA: The buffer for this enzyme contains BSA, so we do not recommend adding more BSA.

Glycerol: The addition of glycerol has not been tested.

DMSO, Betaine: From the preliminary experiments, we believe this enzyme can be used with these additives However, we cannot provide detailed information, such as a recommended concentration, at present.