

## Encyclo polymerase

Encyclo polymerase is a specially developed mix of proofreading and highly processive PCR enzymes and hot start antibodies that inhibit polymerase activity at room temperature, preventing nonspecific amplification and allowing flexible reaction setup. It produces high yields of PCR products from a wide variety of templates and is suitable for most PCR and primer extension applications, including the amplification of difficult templates and long PCR. It is especially recommended for cDNA amplification due to optimal combination of high fidelity and processivity provided by Encyclo polymerase mix.

### Applications:

- cDNA amplification
- Long PCR (up to 15 Kb)
- Amplification of low-copy-number targets
- Routing PCR, real-time PCR with intercalating dyes (SYBR Green I etc.)

Cat #	Product Size	Components
PK002S	200 x 25 $\mu$ l reactions	50X Encyclo polymerase mix, 100 $\mu$ l; 10X Encyclo Plus buffer, 600 $\mu$ l
PK002L	1000 x 25 $\mu$ l reactions	50X Encyclo polymerase mix, 5x100 $\mu$ l; 10X Encyclo Plus buffer, 5 x 600 $\mu$ l

**Shipping/Storage:** Shipping on dry ice or at  $-20^{\circ}\text{C}$ . Once arrived, the kit must be kept at  $-20^{\circ}\text{C}$ .

### Encyclo polymerase features

- High processive 5'>3' DNA polymerase activity
- Proofreading 3'>5' exonuclease activity
- Fast antibody-based hot start
- TA cloning compatibility

### Possible limitations:

- not suitable for real-time PCR methods based on DNA probe breakdown (such as TaqMan) due to the lack of 5'>3' exonuclease activity
- not recommended for allele-specific SNP detection due to the presence of proofreading 3'>5' exonuclease activity

### Reaction Setup:

Prepare a PCR master mix by combining the following reagents in the order shown below, aliquot it into reaction tubes and add missing components, i.e. components that vary from one reaction to the other. All components should be mixed and centrifuged prior to use. If your thermal cycler is not equipped with a heated cover, overlay each reaction with a drop of molecular biology grade mineral oil.

Component	25 $\mu$ l reaction	Final concentration
Sterile water	to 25 $\mu$ l	-
10X Encyclo Plus buffer	2.5 $\mu$ l	1X
50X dNTP mix	0.5 $\mu$ l	1X (0.2 mM each)
Upstream primer	variable	0.2 - 0.5 $\mu$ M
Downstream primer	variable	0.2 - 0.5 $\mu$ M
DNA template	variable	1-200 ng
50X Encyclo polymerase	0.5 $\mu$ l	1X

**Encyclo polymerase** mix contains hot start antibodies that inhibit polymerase activity at room temperature, preventing non-specific amplification and allowing flexible reaction setup. During first denaturation step the antibody is quickly inactivated and PCR proceeds.

## Cycling conditions

Stage	Cycle number	Temperature	Time
Initial denaturation	1	92-95°C	1 - 3 min
Denaturation		92-95°C	5 sec - 1 min
Annealing	10-38	T <sub>m</sub> (55-68°C)	5 sec - 1 min
Extension		72°C	1 min for 1 kb
Final extension (optional)	1	T <sub>m</sub> (55-68°C) 72°C	5 sec - 1 min 2-3 min

T<sub>m</sub> – primer melting temperature.

## Cycling recommendations

- Initial denaturation up to 2-3 minutes is recommended for complex genomic DNA. In other cases, time of initial denaturation can be reduced to 0.5-1 minute.
- Optimal primer annealing temperature depends on the primer structure. Typically annealing temperatures range between 55°C and 72°C. Simplified formula for estimating annealing temperature (T<sub>m</sub>) is  
$$T_m (^{\circ}\text{C}) = 2 \times (\text{A}+\text{T}) + 4 \times (\text{G}+\text{C}).$$
Optimal annealing temperatures may be above or below the estimated T<sub>m</sub> for up to 5°C. In many cases, use of an annealing temperature, which is 5°C above the calculated T<sub>m</sub>, i.e. T<sub>m</sub> + 5°C, can sharply increase PCR specificity.
- To achieve maximal reaction specificity, use primers designed to have a high annealing temperature (preferably 65°C-68°C). Whenever possible, design primer pairs with similar T<sub>m</sub> values. When two primers have different T<sub>m</sub>, use the lowest one for PCR cycling.
- Extension time depends of DNA fragment length (1 min per 1.5 kb). Final extension can be used to ensure full-length polymerization and good yield of the target DNA. This step also increases TA cloning efficiency.
- Decrease the number of PCR cycles if possible. An excessive cycle number is not recommended as nonspecific bands start to appear.

## **TA-cloning of PCR products**

The PCR products generated using Encyclo polymerase contain dA overhangs at the 3' ends and can be cloned into TA vectors. Use freshly amplified PCR product. After the end of PCR, keep the tubes with the PCR products on ice. Using of not purified PCR product critically reduces cloning efficiency so clean-up the amplified DNA using a PCR-column purification protocol or by phenol extraction and ethanol.

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