

DNA Cycle Sequencing Kit

DNA sequencing based on fluorescent-labeled primers

Sequencing Kit

| Cat.-No. | Amount |
|----------|---------------|
| PCR-401S | 100 reactions |
| PCR-401L | 500 reactions |

For *in vitro* use only
 Quality guaranteed for 12 months
 Store at -20°C, avoid frequent thawing and freezing

Terminator A (blue cap)
 dNTP mix containing ddATP

Terminator C (blue cap)
 dNTP mix containing ddCTP

Terminator G (blue cap)
 dNTP mix containing ddGTP

Terminator T (blue cap)
 dNTP mix containing ddTTP

Cycle sequencing polymerase (red cap)
 4 units/μl

Cycle sequencing buffer (green cap)
 10x concentration

Stop solution (purple cap)
 95% formamide containing EDTA, bromophenol blue,
 and xylene cyanol FF

PCR grade water (white cap)

Description

DNA Cycle Sequencing Kit is designed for DNA sequencing based on the Sanger Method (dideoxy chain termination method). It provides a powerful tool to derive rapidly DNA and gene sequence information as required in a multitude of molecular biological and biotechnological applications.

The performance of the kit is based on a modified Taq polymerase showing an equal capability of incorporating ddNTPs and dNTPs. This guarantees the generation of uniform and easy to read sequence band patterns at lowest background. A minimal band compression of GC-rich DNA regions is achieved by optimally balanced termination mixtures in combination with the provided buffer system.

The reaction chemistry of the kit is optimized for automated DNA sequencers and requires fluorescent-labeled primers.

Cycle sequencing

DNA cycle sequencing is a core technique in molecular biology allowing analysis of fmol-quantities DNA template. The enzymatic dideoxy chain termination method of Sanger relies on the linear amplification of a single-stranded template DNA using a single primer and thermostable polymerase. The synthesis of the complementary DNA strand starts at the specific priming site and ends with the incorporation of a chain-terminating dideoxynucleotide triphosphate (ddNTP). This generates a multitude of fragments terminated within the desired length of the sequence. By using the four different ddNTPs in four separate reaction vials, a set of extended primer strands terminated at each A, C, G, and T are obtained. When these fragments are separated on a suitable gel matrix the sequence information can be read from the order of the bands.

Labeled Primers

The kit is optimized for cycle sequencing using fluorescent-labeled primers. The required 5'-end fluorescent label of the primer depends on the optical set-up of the used sequencing machine.

Primers should typically be 20-25 nucleotides in length with a content of 50-60% G+C. They should be checked to avoid forming of internal duplexes or

mispriming to other sites of the template. Minimize the exposure of fluorescent-labeled primers to light.

Premix

First prepare the following premix in a microcentrifuge tube:

| | | |
|-------------------------------------|----------------------------|-----------|
| 4 µl | 10x Sequencing Buffer | green cap |
| 1-2 pmol | fluorescent-labeled Primer | |
| 50-250 fmol or 30-150 ng per kbp | DNA | |
| 1 µl | Sequencing Polymerase | red cap |
| Fill up to 20 µl | PCR grade H ₂ O | white cap |

Mix by pipetting up and down several times.

Recommended assay preparation

- 1) Transfer 4 µl of each Terminator A, C, G, and T (blue caps) into four separate and correspondingly marked tubes
- 2) Add 4 µl of the Premix to each tube and mix gently

Recommended cycling conditions

Place the tubes in the thermal cycler and start the cycling program. The following parameters are recommended:

| | | | |
|----------------------|------|--------|--------|
| Initial denaturation | 95°C | 2 min | 1x |
| Denaturation | 95°C | 30 sec | 20-30x |
| Annealing | 60°C | 30 sec | |
| Elongation | 72°C | 60 sec | |

The annealing temperature depends on the primers used and should be 5-10°C lower than its melting temperature. The melting temperature can be calculated for primers of up to 25 nucleotides using the formula:

$$T_m = 2(A+T) + 4(G+C)$$

A, T, G, C - number of respective nucleotides

For optimal results an empirical optimization of the recommended parameters may be necessary for each new primer/template combination.

Analyzing the samples

- 1) After cycling add 4 µl Stop Solution (purple cap) to each of the vials and mix again
- 2) If the samples cannot be analyzed immediately, they may be stored at -20°C for up to one week
- 3) Incubate the samples at 90°C for 2 min to denature the DNA
- 4) Load 3-5 µl of each reaction onto the gel

Related products

Fluorescent-labeled Oligonucleotides
 Custom Oligonucleotides
 Standard Primers
 Sequencing Pol
 Dideoxynucleotides (ddNTPs)
 Sequencing Service

For further information please visit
www.jenabioscience.com/pcr

Sanger et al. (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**:5463.