

## DNA Cycle Sequencing Kit

### DNA sequencing based on fluorescent-labeled primers

#### Sequencing Kit

	<b>Cat.-No.</b>	<b>Amount</b>
	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/ $\mu$ l

#### **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 specifically engineered 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 containing 7-deaza-dGTP.

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

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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 <sub>2</sub> 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](http://www.jenabioscience.com/pcr)

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