Datasheet

DNA Cycle Sequencing Kit - Sequencing with fluorescent labeled primers by Sanger Method

Cycle Sequencing of cDNA - for Geneom Research

Description: The Maximo-DNA Cycle Sequencing Kit 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 enhanced Taq polymerase showing an equal capability of incorporating ddNTPs and dNTPs. As a result the Maximo-Cycle Sequencing-Kit offers uniform and easy to read sequence band patterns at lowest background.

An absolutely minimal band compression of GC-rich DNA regions is realized by optimally balanced termination mixtures containing 7-deaza-dGTP. The reaction chemistry of the kit is optimized for automated DNA sequencers and requires labelled primers with fluorescent dyes.

Content:

Terminate solution A (blue cap): dNTP mix containing ddATP
Terminate solution C (blue cap): dNTP mix containing ddCTP
Terminate solution G (blue cap): dNTP mix containing ddGTP
Terminate solution T (blue cap): dNTP mix containing ddTTP

Cycle sequencing Polymerase (red cap): 4 Units/µl

Cycle sequencing Buffer (green cap): 10 fold

PCR-grade water (white cap)

Stop-solution (purple cap): 95 % formamide containing EDTA, bromophenol blue, and xylene cyanolFF

Shipping: shipped on blue ice

Storage Conditions: store at -20°C

Note: avoid multiple freeze / thaw cycles

Shelf Life: 18 months

Protocol:

Amount	Component	Codeing
4 µl	10x Sequencing Buffer	green cap
1-2 pmol	fluorescent labeled Primer	-
500-250 fmol or 30-150 ng/kb	DNA	-
1 µl	Sequencing Polymerase	red cap
fill up to 20 µl	PCR-grade water	white cap

Mix by pipetting up and down several times.

Recommended assay preparation:

1) Transfer 4µl of each Terminator A, C, G and T (blue caps) into four separate and correspondingly marked tubes

2) Add 4µl 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	20-30x
elongation	72°C	60 sec	20-30x

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: Tm=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

Comments:

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 dideoxynucleoside 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.

Labelled Primers:
The kit is optimized for cycle sequencing using fluorescent-labelled 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 fluorenscent-labelled primers to light.

Ordering information:

Cat.-no	Description	Amount
S510	MAXIMO DNA Cycle Sequencing Kt	100 rcs
S510L	MAXIMO DNA Cycle Sequencing Kt	500 rcs