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Sanger Sequencing Kit

Product Number: SGS3102

Components

Component	Volume	Storage
Sanger Dye ready reaction mix	50µl	-15 to -25℃
5x Sequencing buffer	250µl	-15 to -25℃

Note:1)Avoid excess (that is, no more than 5-10) freeze-thaw cycles.

2)Before each use of the kit, allow the frozen stocks to thaw at room temperature (do not heat).

3)Mix each stock thoroughly and then centrifuge briefly to collect all the liquid at the bottom of each tube.

4)Whenever possible, keep thawed materials on ice during use. Do not leave reagents at room temperature for extended periods.

Description

Sanger sequencing, also known as chain termination sequencing, is a method of DNA sequencing developed by Frederick Sanger and colleagues in 1977. Sanger sequencing is the gold standard for both first-generation and next-generation sequencing. It is currently the international standard for all gene testing, including fluorescence quantitative PCR TaqMan probe method, ordinary PCR method, chip method, second-generation sequencing method, mass spectrometry method, and other methods.

The Sanger sequencing kit provides the required reagent components for the sequencing reaction in a ready reaction, pre-mixed format. You need only provide your template and the template-specific primer.

These reagents are suitable for performing fluorescence-based cycle sequencing reactions on single-stranded or double-stranded DNA templates, on polymerase chain reaction (PCR) fragments, and on large templates (for example, BAC clones).

Protocol

1. Template Quantity

The table below shows the amount of template to use in a cycle sequencing reaction.

If possible, quantitate the amount of purified DNA by measuring the absorbance at 260 nm or by some other method.

Template	Quantity
PCR product:	
100-200 bp	1-3 ng
200-500 bp	3-10 ng
500-1000 bp	5-20 ng
1000-2000 bp	10-40 ng
>2000 bp	20-50 ng
Single-stranded	25-50 ng
Double-stranded	150-300 ng
Cosmid, BAC	0.5-1.0 µg
Bacterial genomic DNA	2-3 µg

Note:To obtain clean sequencing data, we recommend the following purification methods:

2. Cycle Sequencing

To prepare the reaction mixtures:

Reagent	Quantity
Sanger Dye ready reaction mix	8.0µl
Template	See the table in "Template Quantity"
Primer	3.2pmol

Deionized water	To 20µl

Mix well and spin briefly.

The 5X Sequencing buffer is supplied at a 5X concentration. If you use it for sequencing reactions, be sure the final reaction volume is at a concentration of 1X. For example, for a reaction in 20µl final volume, you woµld use 1µl of Sanger Dye ready reaction mix and 3.6µl of 5X Sequencing buffer as shown below.

Reagent	Concentration	volume	Volume
Sanger Dye ready reaction mix	2.5X	0.8µl	0.2µl
5x Sequencing buffer	5X	3.6µl	0.9µl
Template	-	See the table in "Template Quantity"	See the table in “Template Quantity"
Primer	-	3.2pmol	0.8pmol
Deionized water	-	To 20µl	To 5µl
Final Volume	1X	20µl	5µl

Mix well and spin briefly.

Purifying Extension Products

The best resµlts are obtained when unincorporated dye terminators are completely removed prior to electrophoresis. Excess dye terminators in sequencing reactions obscure data in the early part of the sequence and can interfere with basecalling.

To obtain clean sequencing data, we recommend the following purification:

Ethanol/EDTA Precipitation

1. Remove the 96-well reaction plate from the thermal cycler and briefly spin.
2. Add 5 µl of 125 mM EDTA to each well.
3. Add 60 µl of 100% ethanol to each well.
4. Seal the plate with aluminum tape and mix by inverting 4 times.
5. Incubate at room temperature for 15 min.
6. With the centrifuge use a plate adapter and spin the plate at the maximum speed as follows:
7. 1400-2000×g for 45 min or 2000-3000×g for 30 min
8. Invert the plate and spin up to 185×g, then remove from the centrifuge.
9. Add 60 µl of 70% ethanol to each well.
10. With the centrifuge set to 4°C, spin at 1650×g for 15 min.
11. Invert the plate and spin up to 185×g for 1 min, then remove from the centrifuge.
12. To continue, resuspend the samples in injection buffer.
13. To store, cover with aluminum foil, and store at 4°C.

Ethanol/EDTA/Sodium Acetate Precipitation

1. Remove the 96-well reaction plate from the thermal cycler and briefly spin.
2. Add 2 µl of 125 mM EDTA to each well.
3. Add 2 µl of 3 M sodium acetate to each well.
4. Add 50 µl of 100% ethanol to each well.
5. Seal the plate with aluminum tape and mix by inverting 4 times.
6. Incubate at room temperature for 15 min.
7. With the centrifuge use a plate adapter and spin the plate at the maximum speed as follows:
8. 1400-2000×g for 45 min or 2000-3000×g for 30 min
9. Invert the plate and spin up to 185×g, then remove from the centrifuge.
10. Add 70 µl of 70% ethanol to each well.

11. With the centrifuge set to 4C, spin at 1650×g for 15 min.
12. Invert the plate and spin up to 185×g for 1 min, then remove from the centrifuge.
13. To continue, resuspend the samples in injection buffer.
14. To store, cover with aluminum foil, and store at 4°C.

Keywords

Sanger Sequencing kit, big dye kit.