

Sequenase 7-deaza-dGTP DNA Sequencing Kit

Product Number 70990

Brief Protocol

1. Denature double-stranded templates.

2. Annealing Reaction:

DNA (0.5-1pmol)	__ μ l (up to 7 μ l)
H ₂ O	__ μ l (to adjust total volume)
Sequenase™ reaction buffer	2 μ l
Primer (0.5-2pmol)	1 μ l
Total	10μl

Anneal by heating 2 minutes at 65°C, then cool slowly to <35°C over 15-30 minutes. Chill on ice for use in step 7.

3. While cooling, label, fill and cap tubes with 2.5 μ l of each termination mix. Keep covered at room temperature for steps 5 and 7.

G (2.5 μ l)	A (2.5 μ l)
T (2.5 μ l)	C (2.5 μ l)

4. Dilute labeling mix 1:5 to working concentration. Retain for use in step 6.

Labeling mix	__ μ l (typically 2 μ l)
H ₂ O	__ μ l (typically 8 μ l)

5. Pre-warm 4 termination tubes from step 3 ('G', 'A', 'T' and 'C') in a 37°C bath.

6. Labeling Reaction

To ice-cold annealed DNA mixture (10 μ l), add:

Dithiothreitol (DTT), 0.1M	1 μ l
Diluted labeling mix	2 μ l
[α - ³⁵ S]dATP or [α - ³³ P]dATP	0.5 μ l
Diluted Sequenase polymerase*	2 μ l
Total	15.5μl

Mix and incubate at room temperature 2-5 minutes.

7. Termination Reactions

Transfer 3.5 μ l of the labeling reaction to each termination tube ('G', 'A', 'T' and 'C'), mix and continue incubation of the termination reactions at 37°C for 5 minutes.

8. Stop the reactions by adding 4 μ l of stop solution.

9. Heat samples to 75°C for 2 minutes immediately before loading onto sequencing gel.

***Important:** The Sequenase DNA polymerase must be diluted 8-fold prior to use. If desired, the polymerase for the complete kit can be pre-diluted as follows:

Sequenase DNA polymerase (70775)	25 μ l
Pyrophosphatase (70950)	25 μ l
Glycerol enzyme dilution buffer (70799)	150 μ l

Using this dilution will necessitate the use of a Glycerol Tolerant Sequencing Gel (see protocol book). The polymerase may also be diluted as needed by mixing 1 μ l polymerase with 6 μ l pyrophosphatase and 6 μ l of enzyme dilution buffer (70765). Polymerase diluted this way may be used for ordinary Tris-Borate-EDTA (TBE) gels.

Refer to Protocol Booklet, 70990A, for detailed information on licensing and patents.

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