Sequenase Version 2.0
DNA Sequencing Kit

Product Number 70770

Brief Protocol

1. Denature double-stranded templates.
2. **Annealing reaction**:

DNA (approx. 0.5pmol)	_µl	(up to 7µl)
H_2O	_µl	(to adjust total volume)
Sequenase™ Reaction Buffer	2µl	
Primer (0.5pmol)	1µl	
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 6.
3. While cooling, label, fill and cap tubes with 2.5µl of each termination mix. Use mixes from **red-capped** tubes for dGTP or **orange-capped** tubes for dITP. Keep covered at room temperature for steps 5 and 7.

G (2.5µl)	A (2.5µl)
T (2.5µl)	C (2.5µl)

4. Dilute labeling mix 1:5 to working concentration, dGTP (**green-capped** tube) **or** dITP (**yellow-capped** tube). Retain for use in step 6.

Labeling mix	_µl	(typically 2µl)
H_2O	_µl	(typically 8µl)

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 (10µl), add:

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

   Mix and incubate at room temperature 2-5 minutes.

7. **Termination reactions**

   Transfer 3.5µl 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 4µl 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)	25µl
Pyrophosphatase (70950)	25µl
Glycerol Enzyme Dilution Buffer (70799)	150µl

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 1µl polymerase with 0.5µl pyrophosphatase and 6.5µl of enzyme dilution buffer (70765). Polymerase diluted this way may be used for ordinary Tris-Borate-EDTA (TBE) gels.

Printed in the United States

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26111 Miles Road
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96-0770B/00/10

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