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Product Information

C-24 Sequencing Primer

Catalog Number **P7957**
Store at −20 °C

Product Description
Nucleotide sequence:
5'-OH-CTG-TAT-CAG-GCT-GAA-AAT-CTT-CTC-3'-OH'

C-24 Sequencing Primer is a chemically synthesized, 24 base, single strand oligodeoxyribonucleotide designed for double-stranded or single-stranded DNA sequencing of FLAG® fusion proteins at the C–terminus of *E. coli* pFLAG expression vectors.

Expression Vector	Complimentry to minus strand Map position
pFLAG-1	241 to 264
pFLAG-2	181 to 204
pFLAG-MAC	200 to 223
pFLAG-ATS	260 to 283
pFLAG-CTC	200 to 223
pFLAG-CTS	255 to 278
pFLAG-SHIFT$_{12}$	263 to 286
pFLAG-SHIFT$_{12c}$	203 to 226

The C-24 Sequencing Primer is supplied at an initial concentration of 5 µM in 1X TE buffer (10 mM Tris, 1.0 mM EDTA, pH 8.0).

Reagents Required but Not Provided

- 1X TE buffer
- 5 M NaOH
- 3 M potassium/5 M acetate: To 60 ml 5 M potassium acetate add 11.5 ml glacial acetic acid and 28.5 ml H_2O. The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate.
- Isopropanol

Procedure

A. Preparation of Denatured pFLAG-1 DNA Template
(For sequencing single-stranded DNA, proceed to Section B.)

The following protocol is designed to allow sufficient template for two sets of 4 sequencing reactions (2G, 2A, 2T and 2C reactions).

1. Denature 7 micrograms of highly purified pFLAG-1 supercoiled DNA in 70 µl of 1X TE by adding 3 µl (1/25 volume) of 5 M NaOH and incubating at 37°C for 5 minutes.

2. Precipitate the denatured pFLAG-1 supercoiled DNA for 30 minutes at room temperature by adding 150 µl (2 volumes) of 3 M potassium/5 M acetate : isopropanol (1:3 ratio mixture).

3. Collect the precipitated and denatured pFLAG-1 DNA by centrifugation at 10,000 x g for 5 minutes.

4. Wash the pFLAG-1 DNA pellet with 1 ml ethanol and dry.

5. Resuspend the pFLAG-1 DNA in 20 µl of 1X TE. The final concentration should be 0.35 µg/µl.

The irreversibly denatured pFLAG-1 DNA template can be stored at -20°C at this point if desired.

B. Priming pFLAG-1 DNA Template with C-24 Sequencing Primer

The following protocol uses 10 µl or half of the preceding preparation of irreversibly denatured pFLAG-1 DNA template. This is sufficient for one set of 4 sequencing reactions (G, A, T and C).

1. Dilute a 3 µl aliquot of the C-24 Sequencing Primer with 6 µl of 1X TE to make a final concentration of 1.67 pmol/µl.

2. Add 2-3 µl (3.5-5 pmoles) of C-24 Sequencing Primer to 10 µl (1 pmole) of denatured pFLAG-1 DNA template.

3. To 12-13 µl of C-24 primer/pFLAG-1 DNA template add an appropriate volume of sequencing buffer to make the buffer concentration 1X. Heat at 70°C in a wet temperature block for 2 minutes.

4. Slowly cool the mixture to 45°C by placing the temperature block at room temperature for about 20 minutes.

5. Distribute the C-24 primer/pFLAG-1 DNA template to four tubes to be used in the G, A, T and C DNA sequencing reaction.

The C-24 primer/pFLAG-1 DNA template is now ready for supercoil sequencing of the DNA sequence corresponding to the C-terminal FLAG fusion junction.

The DNA sequence corresponding to the fusion junction will be approximately 10-45 bases away from the 3' end of the C-24 sequencing primer reading from the bottom of an autoradiogram, depending on where the insert was cloned in the MCS.

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