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Product Information

C-CMV-24 Sequencing Primer

Catalog Number **P5475**
Store at 0 to –20 °C

Product Description
Nucleotide sequence:
5'-TAT-TAG-GAC-AAG-GCT-GGT-GGG-CAC-3'

C-CMV-24 sequencing primer is a chemically synthesized, 24 base, single-strand oligodeoxy-ribonucleotide designed for double-strand or single-strand DNA sequencing at the C-terminus of inserts in the pFLAG-CMV™ expression vectors.

The primer is complementary to map position nucleotides 1116-1139 of negative, non-coding DNA strand of the pFLAG-CMV-1 vector and nucleotides 1073-1096 of negative, non-coding DNA strand of the pFLAG-CMV-2 vector.

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

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-CMV 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 2 C reactions).

Denature 7 micrograms of highly purified pFLAG-CMV 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.

1. Precipitate the denatured pFLAG-CMV 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).

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

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

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

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

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

The following protocol uses 10 μl or half of the preceding preparation of irreversibly denatured pFLAG-CMV 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-CMV-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-CMV-24 sequencing primer to 10 μl (1 pmole) of denatured pFLAG-CMV DNA template.

3. To 12-13 μl of C-CMV-24 primer/pFLAG-CMV 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-CMV-24 primer/pFLAG-CMV DNA template to four tubes to be used in the G, A, T and C DNA sequencing reactions.

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

   The DNA sequence corresponding to the fusion junction will be >58 bases away from the 3' end of the C-CMV-24 sequencing primer reading from the bottom of an autoradiogram.

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