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Product Information

N-26 Sequencing Primer

Catalog Number **P7832**
Store Temperature –20 °C

Product Description

Nucleotide sequence:
5′-OH-CAT-CAT-AAC-GGT-TCT-GGC-AAA-TAT-TC-
-3-OH′

The N-26 sequencing primer is a chemically synthesized, 26 base, single strand oligodeoxy-ribonucleotide designed for double-stranded or single-standed DNA sequencing of FLAG® fusion junctions corresponding to the N-terminus of FLAG fusion proteins expressed by *E. coli* pFLAG expression vectors. The N-26 primer is especially useful for sequencing N-terminal FLAG fusion junctions encoded by inserts cloned into the pFLAG-MAC™, pFLAG-ATS™, pFLAG-1, pFLAG-2, pFLAG-Shift™$_{12}$, and pFLAG-Shift$_{12c}$ N-terminal expression vectors. For all pFLAG expression vectors, the N-26 primer is complementry to the plus strand map positions 1 to 26.

The N-26 sequencing primer is supplied at an initial concentration of 5 μM in 0.1× TE buffer (1 mM Tris, pH 8.0, 0.1 mM EDTA).

Reagents Required but Not Provided

- 1× TE buffer (10 mM Tris, pH 8.0, 1.0 mM EDTA)
- 5 M NaOH
- 3 M potassium/5 M acetate: To 60 ml of 5 M potassium acetate add 11.5 ml of glacial acetic acid and 28.5 ml of water. 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 1× 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 × *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 1× 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 N-26 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 N-26 sequencing primer with 6 µl of 1× TE to make a final concentration of 1.67 pmol/µl.
2. Add 2-3 µl (3.5-5 pmoles) of N-26 sequencing primer to 10 µl (1 pmole) of denatured pFLAG-1 DNA template.
3. To 12-13 µl of N-26 primer/pFLAG-1 DNA template add an appropriate volume of sequencing buffer to make the buffer concentration 1×. 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 N-26 primer/pFLAG-1 DNA template to four tubes to be used in the G, A, T and C DNA sequencing reaction.

The N-26 primer/pFLAG-1 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 approximately 150 bases away from the 3′ end of the N-26 sequencing primer reading from the bottom of an autoradiogram.

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