TopoGEN
Products and Services for **Cancer Research.**

Supercoiled pHOT-1 DNA

Relaxed pHOT-1 DNA

Product Description

This plasmid is ideal for assaying any topoisomerase. pHOT-1 is relatively small (<3KB) which is ideal for displaying intermediate topoisomers. Since all topoisomerases display degenerate DNA binding and cleavage sequences, pHOT-1 will effectively support activity for a wide array of topoisomerase. Supercoiled pHOT-1 is a scaled-down derivative of pBR322 and optimized for higher yields. It has one additional feature for researchers who wish to assess human topo I cleavage at a single sequence: we have included the human topoisomerase I preferential binding site (the hexadecameric sequence described by Westergaard and co-workers, see Bonven et al., 1985) that is derived from the tetrahymena ribosomal gene repeat. This construct is useful as a topo I cleavage substrate since the enzyme efficiently cleaves at the hexadecameric site in the absence of camptothecin. This allows the investigator to rigorously evaluate new potential topo I inhibitors because a single background cleavage is a built-in control; if the agent stimulates cleavage at this and/or any other sequence in the fragment that contains the hexadecameric site, prominent cutting is readily revealed. Typically, it is difficult to trap topo I cleavages on DNA; one must use more enzyme since cleavage complexes consume the enzyme stoichiometrically. For this reason, higher levels of enzyme are required for analysis of DNA cleavages. The fragment can be isolated from the polylinker using the sites shown below (protocols in Maniatis cloning book), end labeled and used as a cleavage substrate.

Relaxed pHOT-1 is suited to assaying the supercoiling activity of DNA gyrase. It is also useful for assaying alterations in DNA linking number for assessing intercalation. Relaxed pHOT-1 DNA is prepared using high purity topo I relaxation reactions, followed by inactivation and re-purification of the relaxed product. TopoGEN staff then verify the relaxation status of each lot and validates that the substrate can be supercoiled by *E. coli* and *S. aureus* DNA gyrase.

Quality Control Analysis

•Purity was determined spectroscopically using A260/A280 ratio method. Incubation under conditions optimal for Gyrase activity did not result in any detectable conversion of the DNA to nicked or linear DNA forms.
•For both supercoiled and relaxed DNA substrates greater than 85% of the DNA will be supercoiled (form I) DNA the remainder as nicked open circular (form II) DNA. There are no detectable linear DNA forms.

Storage Buffer

All DNAs are stored in TE buffer (10 mM Tris-Cl (pH 7.5), 1 MM EDTA) at the concentration specified in the above upper right-hand corner.

Storage and Shipping Conditions

These DNAs should be stored at 4°C. The DNA is shipped at ambient temperature (or may be shipped on dry ice with enzyme shipments).

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