

# *pHOT1- DNA*

## *Catalog Number:*

- 2030-2 Supercoiled pHOT1 DNA (20 ug)**  
 **2035-1 Relaxed pHOT1 DNA (10 ug)**



## *Description:*

The plasmid pHOT1 contains the high affinity topo I cleavage 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. For the latest map showing sequence of the topo I site, go on line to [www.topogen.com](http://www.topogen.com) and navigate to "DNA Substrates" and locate pHOT1 page.

## *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).

## *References*

- Bonven et al., Cell 41:541 (1985)  
Sambrook et al. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Press, Cold Spring Harbor, NY

## *Product Application and Disclaimer*

This product is not licensed or approved for administration to humans or animals. It may be used with experimental animals only. The product is for in vitro research diagnostic studies only. The product is non-infectious and non-hazardous to human health. This information is based on present knowledge and does not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship. TopoGEN, Inc. shall not be held liable for product failure due to mishandling and incorrect storage by end user. TopoGEN's liability is limited to credit or product replacement.

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