

Positively Supercoiled pBR322



Product Description (#POS5001)

Supercoiled plasmid pBR322 DNA is produced by the large-scale alkaline-lysis method (Sambrook *et al.*, 1989) from a high-copy number derivative of pBR322 (Boros *et al.*, 1984) and is relaxed using chicken erythrocyte topoisomerase I (Trask and Muller, 1983).

It is then treated in the presence of excess DNA gyrase to positively supercoil it, phenol/chloroform extracted and suspended in TE to a final concentration of 1 mg/ml

Store at 4°C

For *in vitro* laboratory research use only

TE Storage Buffer

10 mM Tris.HCl (pH 7.5)
1 mM EDTA

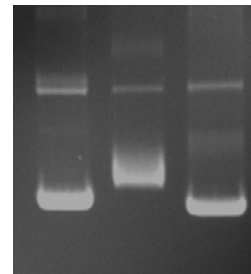
Incubation Conditions

Relaxed pBR322 incubated with 100X excess of DNA gyrase at 37°C for 3 hrs in 35 mM Tris.HCl pH 7.5, 24 mM KCl, 4 mM MgCl₂, 1.8 mM spermidine, 2 mM DTT, 6.5 % (w/v) glycerol and 0.36 mg/ml BSA is completely converted to the positively supercoiled form as assessed by agarose gel electrophoresis +/- chloroquine (CQ).

The mobility of positively supercoiled pBR is slightly greater than negatively supercoiled or relaxed pBR on agarose gels containing CQ.

- 1) Negatively supercoiled pBR322
- 2) Relaxed pBR322
- 3) Positively supercoiled pBR322

-veSC Rel +ve SC



1% TAE gel + 5 µg/ml CQ

References

Boros, I., Pósfai, G. & Venetianer, P. (1984). High-copy –number derivatives of the plasmid cloning vector pBR322. *Gene* 30, 257-260

Sambrook, J., Fritsch, E.F. & Maniatis, T (1989). *Molecular cloning: a laboratory manual*. Cold Spring Harbor Press. Cold Spring Harbor.

Trask, D. K. & Muller, M. T. (1983) Biochemical characterization of topoisomerase I purified from avian erythrocytes. *Nucleic Acids Res.* 11, 2779-2800