

Supercoiled pBR322



Product Description (Product Numbers #S5001, S2502, S5003 and S1004)

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). It is then further supercoiled by treatment with topo I in the presence of an intercalator.

It is shipped on dry ice at a concentration of 1mg/ml in TE.

Store at 4°C

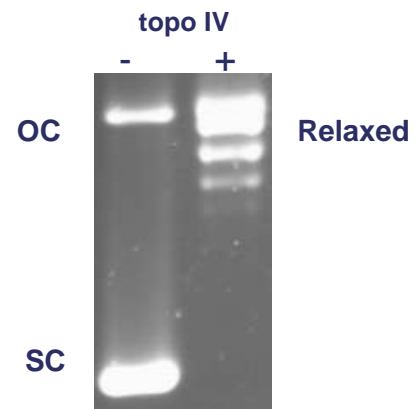
For *in vitro* laboratory research use only.

TE Storage Buffer

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

Relaxation Assay

1 U (in 1µl) of topoisomerase IV will relax 0.4 µg of supercoiled pBR322 when incubated in 1X Assay Buffer in a total reaction volume of 30 µl at 37°C for 30 minutes. Gels are run in the absence of Ethidium Bromide or Chloroquine.



Quality Control

Purity was determined spectroscopically using the ratio between the OD₂₆₀ and OD₂₈₀ readings.

Incubation under gyrase assay conditions (using 5X Assay Buffer) at 37°C for 30 minutes did not result in any detectable conversion of the DNA to either open circular or linear forms when assessed by agarose gel electrophoresis.

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.