Supercoiled pBR322

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

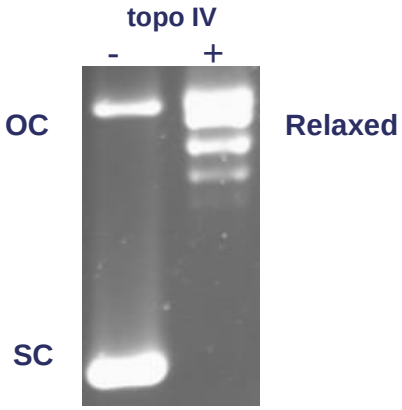
Supercoiled plasmid pBR322 DNA is produced by the large-scale alkaline-lysis method (Sambrook et al., 1989) from a high-copy number derivative of pBR322 (Boros et al., 1984). It is then further supercoiled by treatment with topo I in the presence of an intercalator.
It is shipped on dry ice at a concentration of 1mg/ml in TE.
Store at -20°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 OD_{260} and OD_{280} 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 clonoing 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.