Relaxed pBR322

Product Description (Product Numbers R5001, R2502, R5003 and R1004)

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 topoisomerase I.
It is shipped on dry ice at a concentration of 1 mg/ml in TE.
For *in vitro* laboratory research use only.

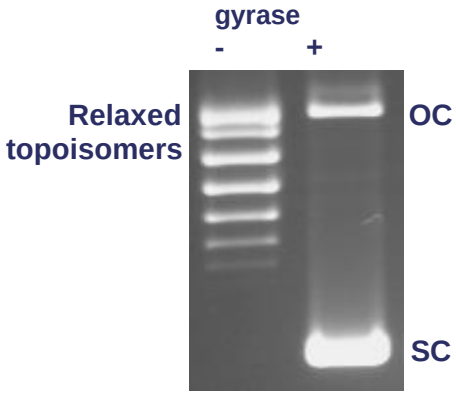
TE Storage Buffer

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

Supercoiling Assay

0.5 µg of relaxed pBR322 when incubated with 1 U of DNA gyrase in a reaction volume of 30 µl at 37 °C for 30 minutes in Assay Buffer is completely converted to the supercoiled form.
Gels are run in the absence of intercalators (e.g. ethidium bromide or chloroquine).

OC=open circular SC=supercoiled



Quality Control

Purity was determined spectroscopically using the ratio between the OD_{260} and OD_{280} readings.

Incubation under gyrase assay conditions 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 Laboratory Press, Cold Spring Harbor, Plainview, NY.