# DNA Unwinding Assay Kit

## Product Description (Product Numbers DUKSR001, DUKR002, DUKS003)

The enzyme is supplied at a minimum concentration of 2 U/µl in Dilution Buffer.
Store at -80 °C. (Stable for 3 months undiluted.) It is recommended that the enzyme is aliquoted to avoid repeated freeze-thaw cycles.
**For *in vitro* laboratory research use only.**

## Dilution Buffer

50 mM Tris.HCl (pH 7.9)
500 mM NaCl
1 mM DTT
1 mM EDTA
50 % (v/v) glycerol

## Assay Buffer (supplied as 2X stock)

50 mM Tris.HCl (pH7.9)
50 mM NaCl
1 mM EDTA
1 mM DTT
20 % (v/v) glycerol

## Unwinding Assay

A typical reaction will contain 15 µl of (2x) Assay Buffer, 0.5 µl of pBR322 (1 µg/µl) plus wheat germ topo I (2.5 U) and compounds as appropriate, in a total volume of 30 µl. The reaction is incubated at 37 °C for 30 minutes. 20 µl of water and 50 µl of butanol are added and the reaction vortexed briefly and centrifuged. After extraction, the aqueous layer (lower) layer is mixed with an equal volume of STEB, extracted with chloroform/isoamyl alcohol and analysed on agarose gels.
Gels can be run in the presence or absence of chloroquine (CQ).
The gel opposite shows the effect of ethidium bromide (EtBr), an intercalator, in the assay using supercoiled pBR322. Topo I alone relaxes the plasmid (lane 3) but in the presence of both topo I and EtBr, the plasmid runs close to supercoiled DNA (lane 4).

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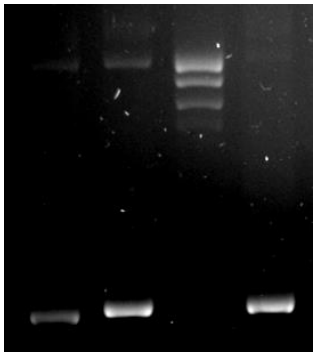
|---|---|---|---|---|
| **wg topo I** | **-** | **-** | **+** | **+** |
| **EtBr** | **-** | **+** | **-** | **+** |



## Quality Control

Endonuclease assay: 0.5 µg relaxed pBR322 incubated with 1 U of wheat germ topo I for 6 hours at 37 °C shows no detectable conversion of relaxed DNA to either open circular or linear forms when assayed by agarose gel electrophoresis.