

Pseudomonas aeruginosa Gyrase



Product Description (PAG1001, PAG5002 and PAG1003)

Pseudomonas aeruginosa gyrase is purified after heterologous expression in *E. coli* and is supplied as an equimolar complex of the subunits. The enzyme is supplied at a minimum concentration of 10 U/μl in Dilution Buffer.

Store at -80°C. It is recommended that the enzyme is aliquoted to avoid repeated freeze-thaw cycles.

For *in vitro* laboratory research use only.

Dilution Buffer (1X)

50 mM Tris.HCl (pH 7.6)
2 mM DTT
50 % (w/v) glycerol
1 mM EDTA
50 mM NaCl

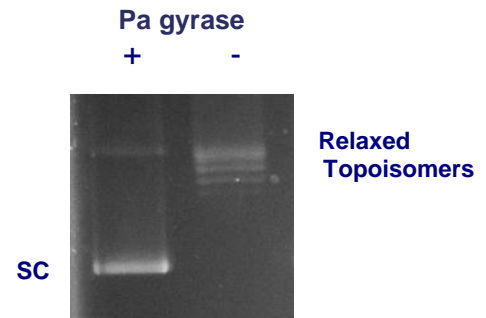
Assay Buffer (supplied as 5X stock)

35 mM Tris.HCl (pH 7.5)
24 mM KCl
4 mM MgCl₂
2 mM dithiothreitol
1.8 mM spermidine
1 mM ATP
6.5 % (w/v) glycerol

Supercoiling Assay

1 U of *Pseudomonas aeruginosa* gyrase is incubated with 0.5 μg of relaxed pBR322 in a reaction volume of 30 μl at 37°C for 30 minutes in Assay Buffer.

Gels are run in the absence of Ethidium Bromide or Chloroquine.



Quality Control

- 1) Purity: *P. aeruginosa* topo gyrase is purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis.
- 2) No activity was detectable when the single subunits were assayed alone
- 3) Supercoiling activity was fully inhibited by ciprofloxacin at 1 μM
- 4) The enzyme was tested for activity in an NADH-linked ATPase enzyme assay and showed activity which was fully inhibited by novobiocin at 1 μM