

S. aureus Gyrase Supercoiling Assay Kit



Product Description (Product numbers SAS4001 and SAS4002)

S. aureus gyrase is purified after heterologous expression in *E. coli* and is supplied as an A₂B₂ complex. The enzyme is supplied at a concentration of 5 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 7.5)
1 mM DTT
1 mM EDTA
40 % (w/v) glycerol

Assay Buffer (supplied as 5X stock)

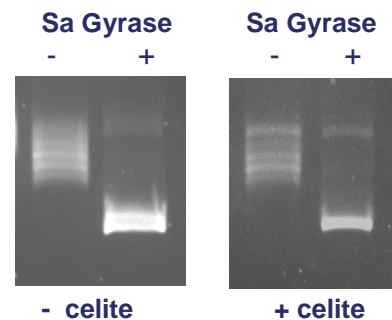
40 mM HEPES. KOH (pH 7.6)
10 mM magnesium acetate
10 mM DTT
2 mM ATP
500 mM potassium glutamate
0.05 mg/ml albumin

Supercoiling Assay

1 U of 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.

Due to the high potassium glutamate content of the Assay Buffer, the following extraction may be performed to improve gel resolution:
After incubation (and before the addition of stop dye), add an equal volume of Celite slurry. Vortex briefly and spin 1 minute.
Remove supernatant and add 100 μl Celite wash buffer.
Vortex briefly and spin for 1 minute. Remove supernatant and resuspend pellet in original volume of stop dye.



Quality Control

1) Purity: *S. aureus* gyrase is purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis. 2) pBR322 was also incubated for 4 hrs in assay buffer + 10 mM MgCl₂ at 37 °C. These tests were negative for the formation of linear products, indicating the absence of nuclease contamination. 3) No activity was detectable when the single subunits were assayed alone