

S. aureus DNA Gyrase Supercoiling Assay Kit



Product Description (Product numbers SAS4001 and SAS4002)

S. aureus DNA gyrase is purified after heterologous expression in *E. coli* and is supplied as an A₂B₂ complex.

The enzyme is supplied at a minimum concentration of 10 U/μl in Dilution Buffer. However, we recommend that the enzyme is titrated into the assay to ascertain the minimum volume of enzyme required per assay to achieve full supercoiling. Particularly if the kit is being used for drug screening purposes. Please refer to the protocol for more information:

<https://www.inspiralis.com/assets/TechnicalDocuments/S.-aureus-Gyrase-Supercoiling-Assay-Protocol.pdf>

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

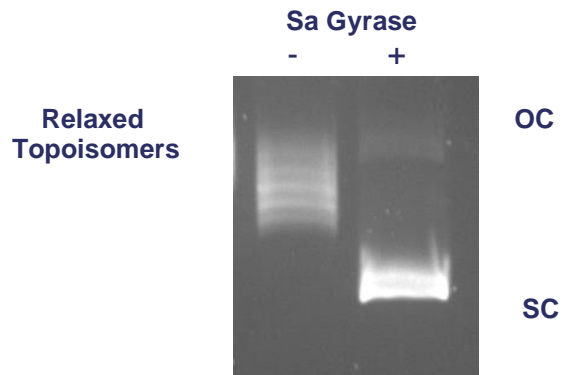
Assay Buffer (supplied as 5X stock)

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 DNA 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: *S. aureus* DNA 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