

# *S. aureus* Gyrase Assay Kit for Cell Extracts



## Product Description (Product Numbers SAGE001, SAGE002)

Contains 5X Assay Buffer, Dilution Buffer, relaxed pBR322 DNA and supercoiled pBR322 marker.  
Store at -20°C.

**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.

