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ProductInformation

RIBONUCLEIC ACID, TRANSFER
tRNA for Coprecipitation

Product No. **R 5636**

Lot 014K6001

Store below 0 °C

PRODUCT SUMMARY

Concentration: 10.0 mg/ml

Endonuclease (Nickase): None detected
DNase: None detected

Suitable for use as carrier in nucleic acid purification and precipitation

COMMENTS

Concentration was deternined based on the assumption that a 40 µg/ml solution of tRNA would have an absorbance of 1.0 at 260 nm.

ENDONUCLEASE (NICKASE)

One µg of pBR322 DNA was incubated with tRNA at a final concentration of 2 µg/µl in a 50 µl reaction mixture containing 30 mM Tris-HCl, pH 7.8, 50 mM NaCl and 10 mM $MgCl_2$ for 16 hours at 37 °C. No conversion of the covalently closed circular DNA to the nicked or linear form was observed by agarose gel electrophoresis. Detection limit: Conversion of 1% of the DNA substrate is detectable.

ENDONUCLEASE-EXONUCLEASE

One µg of λ Hind III fragments was incubated for 16 hours at 37 °C with tRNA at a final concentration of 2 µg/µl in a 50 µl reaction mixture containing 30 mM Tris-HCl, pH 7.8, 50 mM NaCl and 10 mM $MgCl_2$. No degradation of the DNA fragments was detected by agarose gel electrophoresis. Detection limit: Degradation of 10% of the DNA substrate is detectable

SUITABILITY FOR USE AS CARRIER

Hind III-λ DNA at 0.1 µg/ml, 0.5 µg/ml, and 1.0 µg/ml was extracted with phenol/chloroform and precipitated with ethanol as follows: To 500 µl of DNA solutions (in 1.5 ml microcentrifuge tubes) at each concentration 1 ml of phenol/chloroform (1:1) was added. The solutions were then vortexed briefly and microcentrifuged at 15,000 rpm for 1 minute. 400 µl of the upper aqueous phase from each tube were placed in a microfuge tube. To one set of tubes 10 µl of a 10 µg/µl tRNA for carrier were added and to another set no tRNA was added. Each tube was brought to approximately 0.27 M sodium acetate by the addition of 40 µl of a 3 M solution (pH 7.0). Then, 1 ml of 95% ethanol was added to each tube and the tubes were stored at –20 °C overnight. After microcentrifuging for 10 min. the supernatant was aspirated and the pellets were air dried for 2 hours. The pellets were dissolved in 50 µl H_2O and analyzed by agarose gel electrophoresis. By observation of the ethidium bromide stained samples it was found that the addition of carrier tRNA for coprecipitaion improved the recovery of DNA approximately 10-fold.

3/04