DNase I Treatment
Version 1
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Resuspending DNase I from powder

Determine number of units per mg powder: Roche grade II (cat.no. 10 104 159 001) contains approximately 2,000 Kunitz units per mg and comes in 100 mg size which equals 200,000 U. Resuspend in 5ml NEB recommended storage buffer, 10 mM Tris-Cl pH 7.5, 2 mM $CaCl_2$ in 50% glycerol which equals 40,000 U/ml and store at -20^{o}C.

Unit definition

One unit is the amount of enzyme which will completely degrade 1µg of pBR322 DNA in 10 min at 37^{o}C in DNase I reaction buffer.

Reaction Buffer

Prepare a 10x reaction buffer containing $MgCl_2$ and $CaCl_2$ which are required for enzymatic activity. Final 1x buffer contains 10 mM Tris-HCl pH 7.6, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$.

DNase I reaction conditions

DNase I was tested for ability to degrade DNA in solutions containing iron chloride, EDTA-Mg ascorbate buffer and cesium chloride (see BTP Exp. 72) under conditions of 37^{o}C for 1 hr, room temperature for 2 hr and 4^{o}C overnight. The DNA concentration (1 Kbp DNA ladder) in each solution was 200 ng/µl. The DNase I (40,000 U/ml) was diluted 1:40 in 10x reaction buffer with final concentration of 1,000U/ml (=1 U/µl) in 10x buffer. The diluted DNase I was used at a 1:10 dilution in the DNA solutions: 1 µl DNase I in 9 µl DNA. The DNA was degraded in each solution at all the reaction conditions tested.

DNase I Storage Buffer: 10 mM Tris-HCl, pH 7.5, 2 mM $CaCl_2$ in 50% glycerol

100 µl 1 M Tris-HCl, pH 7.5 (autoclaved or filter-sterilized stock)
20 µl 1 M $CaCl_2$ (filter-sterilized stock)
10 ml 50% Glycerol (autoclaved)

10X DNase I Reaction Buffer: 100 mM Tris-HCl, pH 7.6, 25 mM $MgCl_2$, 5 mM $CaCl_2$

1 ml 1 M Tris-HCl, pH 7.5 (autoclaved or filter-sterilized stock)
250 µl 1 M $MgCl_2$ (filter-sterilized stock)
50 µl 1 M $CaCl_2$ (filter-sterilized stock)
8.7 ml Q-water (autoclaved)

DNase Treatment and Inactivation

DNase I made up to 40,000 U/ml (-20°C freezer door) = stock solution.

Dilute stock solution 1:40 in 10x reaction buffer (room 207, center counter, 15ml tube) = 1,000 U/ml = 1 U/ul = working dilution.

Use at a 1:10 dilution: 1µl per 9 µl solution to be treated.

Incubate under one of the following conditions: 4°C, overnight; room temperature for 2 hr; 37°C for 1hr.

Inactivate DNase by adding 100 mM EDTA/100 mM EGTA final concentration:

EDTA disodium salt dehydrate: weigh out 3.72 g and place in 5 ml molecular biology grade water. Add a few NaOH pellets to get to pH 9 and dissolve EDTA (can warm to 45°C with stirring to aid in dissolution). Measure volume and determine molarity: 1M = 3.72g/10 ml, therefore, measured volume/10 ml = final molarity.

Note: EDTA tetrasodium salt dihydrate is somewhat more soluble and can be made more easily to 1.5M. FW is slightly more so weigh out 3.80 g and follow directions above.

EGTA tetrasodium salt: weigh out 4.68 g and place in 5 ml molecular biology grade water. Stir until dissolved. Measure volume and determine molarity: 1M = 4.68g/10 ml, therefore, measured volume/10 ml = final molarity.

Note: EGTA tetratsodium salt is far easier to get into solution than the disodium salt. It is possible to get to 1.5M with this reagent.