Solutions

TE

pH 7.4

10 mM Tris · Cl (pH 7.4)
1 mM EDTA (pH 8.0)

pH 7.6

10 mM Tris · Cl (pH 7.6)
1 mM EDTA (pH 8.0)

pH 8.0

10 mM Tris · Cl (pH 8.0)
1 mM EDTA (pH 8.0)

STE (also called TNE)

10 mM Tris · Cl (pH 8.0)
100 mM NaCl
1 mM EDTA (pH 8.0)

Formamide (Deionized)

Mix 50 ml of formamide and 5 g of mixed-bed, ion-exchange resin (e.g., Bio-Rad AG 501-X8, 20–50 mesh). Stir for 30 minutes at room temperature. Filter twice through Whatman No. 1 filter paper. Dispense into 1-ml aliquots and store at −20°C.

Denhardt's Solution (50 ×)

Ficoll	5 g
polyvinylpyrrolidone	5 g
BSA (Pentax Fraction V)	5 g
H_2O	to 500 ml

Filter through a disposable Nalgene filter. Dispense into 25-ml aliquots and store at −20°C.

10% Bovine Serum Albumin (BSA)

BSA (Pentax Fraction V)	1 g
H_2O	10 ml

Dispense into aliquots. Store at −20°C.

ATP (0.1 M)

Dissolve 60 mg of ATP in 0.8 ml of H_2O. Adjust the pH to 7.0 with 0.1 M NaOH. Adjust the volume to 1.0 ml with H_2O. Dispense the solution into small aliquots and store at −70°C.

Ribo- and Deoxyribonucleotide Triphosphates (~10 mM)

Dissolve NTP or dNTP in water directly in the shipping bottle at an expected concentration 10 mM. Using a dilute solution (0.05 M) of Tris base, an automatic micropipettor, and pH paper, adjust the pH to 7.0. Dilute an aliquot of the neutralized NTP or dNTP appropriately and read the optical density at the wavelengths given in Table A.3. Using the values for the extinction coefficients in the table, calculate the actual concentration. Freeze away in small aliquots at −20°C.

TABLE A.3. OPTICAL DENSITIES OF RIBO- AND DEOXYRIBONUCLEOTIDE TRIPHOSPHATES

Base	Wavelength	Extinction coefficients for bases ϵ (M^{-1} cm^{-1})
A	259	1.54×10^4
G	253	1.37×10^4
C	271	9.1×10^3
U	262	1.0×10^4
T	260	7.4×10^3

For a cell with a 1-cm path length, absorbance = ϵ/M