Stock Solutions

1 Kb Markers
BRL 1kb is a partial digest, and 1.6 kb band is ~10% of total (1.025 µg/µl)
Dilute 1/50 and use 10 µl (~200 ng)
The 1.6 kb band from 10 µl of marker = ~20 ng of DNA

Heat Treated RNase A Aliquots
• Use RNase Type IIA from Sigma (R-5000).
1. Dissolve 100 mg RNase A in 5 mls of: 10 mM Tris, pH 7.5, 1 mM EDTA, 15 mM NaCl
2. Place in boiling H₂O bath for 15 minutes.
3. Cool slowly to room temperature.
4. Add 5 mls of glycerol and mix completely.
5. Store 1 ml aliquots at -20°C.
6. Use sterile pipet tips when removing solution.

Proteinase K Stock Solution Aliquots
• Use Proteinase K from BMB, GIBCO/ BRL, or Sigma.
1. Dissolve 50 mg proteinase K in 0.5 ml 10 mM Hepes-NaOH, pH 7.5, 1 mM CaCl₂.
2. Self-digest 15 minutes at 37°C
3. Add 0.5 ml 50% glycerol.
2. Divide into 10 X 100 µl aliquots. Store at -20°C.
3. Use sterile pipet tips when removing solution.

Tolerates: 1% SDS, 4 M urea, up to 65°C.

Ampicillin Stock Aliquots
1. Dissolve 1.0 g of ampicillin (sodium salt) in 10 mls of ddH₂O in 10 ml cylinder.
2. Filter sterilize with syringe into 10 sterile microfuge tubes. Label “AMP, 1000X.”

Protease Inhibitor Cocktails (Pics)

1000X "D" in DMSO:
0.88 g PMSF (0.5M) Sigma P 7626
10 mg Pepstatin A BMB 1359 053 (10 mg; use all)
10 mg Chymostatin BMB 1004 638 (10 mg; use all)

Add DMSO to 10 mls. Dissolve. Store at -20°C in 1 ml aliquots.

1000X "W" in water:
1.57 g Benzamidine (1M) Sigma B 6506
5 mg Leupeptin Sigma L 2884 (5 mg; use all)
5 mg Bestatin Sigma B 8385 (5 mg; use all)
[5 mg Aprotinin Optional: add until the lab runs out.]

Add ddH₂O to 10 mls. Dissolve. Store at -20°C in 1 ml aliquots.
Stock Solutions

Mountant Medium for Immunofluorescence: 10 mls
10 mg p-phenylene diamine (pDA)
1 ml 100 mM Tris•HCl, pH 8.5 (at 25°C)
9 ml Glycerol
Dissolve the pDA in Tris buffer at room temperature. Add glycerol and mix.
Aliquot as 9 X 1 ml (1.5 ml tubes) + 10 X 100 µl (0.5 ml tubes).
Store at -70°C
(Note: pH must be >8.0. See Biotechniques, 20:398-400, March 1996.)

Vanadyl Adenosine Complex (VAC): 30 mls
1. Prepare 2 M vanadyl sulfate (Aldrich 20,486-2): 1.74 g in 4 ml ddH2O.
2. Prepare 250 mM adenosine (Sigma A-9251): 2 g in 30 ml ddH2O. Heat to near boiling while stirring to dissolve. Once in solution, adjust to 30 ml.
3. To 24 ml adenosine, slowly add 3 ml vanadyl sulfate solution while continuing to heat and stir to prevent precipitation.
4. Adjust pH to 6.0 with 10 N NaOH. Then pH to 7.0 with 1 N NaOH. Solution will clear at pH 6.0 and remain clear. Use pH paper for hot solution.
5. Adjust final volume to 30 ml. Aliquot in 1 ml portions. Keep hot and stirred. Store at -80°C.
   Final concentration in 200 mM.

Protein Assay Reagents

Reagent A1: 500 mls
50 g Na2CO3
10 g NaOH
0.5 g Potassium Tartrate
Add ddH2O to 500 mls
Store in tightly capped plastic bottle.

Reagent A2: 50 mls
0.25 g CuSO4
Add ddH2O to 50 mls
Store in tightly capped plastic bottle.

Tracking Dyes
Bromophenol Blue, 100X, 5% wt./vol. in water
Xylene cyanol FF, 50X, 2.5% wt./vol. in water

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<th>Gel</th>
<th>Percent</th>
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<th>Xylene cyanol FF</th>
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<td>~5 kb</td>
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<tr>
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<td>10%</td>
<td>10 nt</td>
<td>55 nt</td>
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Formamide Loading Solution
95% Formamide, 10 mM EDTA, pH 8, 1X tracking dyes