

Sugden Lab.

## ***Common Reagents for Molecular Cloning***

### **TE**

10 mM Tris-HCl (pH 8.0)  
1 mM EDTA (pH 8.0)

### **Tris-acetate (TAE)** 50 x Stock solution (final:0.04 M Tris-acetate and 0.001 M EDTA)

Tris base 242 g  
Glacial acetic acid 57.1 ml  
0.5 M EDTA (pH 8.0) 100 m  
dH<sub>2</sub>O adjust to final volume of 1L

### **Tris-borate (TBE)** 10 x Stock solution (final:0.045 M Tris-borate and 0.001 M EDTA)

Tris base 54 g  
0.5 M EDTA (pH 8.0) 20 m  
dH<sub>2</sub>O adjust to final volume of 1L

### **5 M NaCl**

NaCl 292.2 g  
dH<sub>2</sub>O adjust to final volume of 1L

1. Dissolve NaCl in 800 ml dH<sub>2</sub>O, heat to 68C to assist dissolution.
1. Adjust to final volume of 1L and sterilize by autoclaving.

### **20% SDS**

SDS (electrophoresis-grade) 100 g  
dH<sub>2</sub>O adjust to final volume of 1L

2. Dissolve SDS in 900 ml dH<sub>2</sub>O, heat to 68°C to assist dissolution.
3. Adjust pH to 7.2 by adding a few drops of concentrated HCl and the final volume to 1L.

### **3 M sodium acetate (pH 5.2)**

sodium acetate.3H<sub>2</sub>O 408.1 g  
dH<sub>2</sub>O adjust to final volume of 1L

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1. Dissolve sodium acetate.3H<sub>2</sub>O in 800 ml dH<sub>2</sub>O, adjust pH to 5.2 with glacial acetic acid
2. Adjust to final volume of 1L and sterilize by autoclaving.

### **10 M Ammonium acetate**

ammonium acetate                      770 g  
dH<sub>2</sub>O      adjust to final volume of 1L

1. Dissolve ammonium acetate in 800 ml dH<sub>2</sub>O, adjust pH to 5.2 with glacial acetic acid
2. Adjust to final volume of 1L and sterilize by filtration.

### **0.5 M EDTA (pH8.0)**

EDTA.2Na.2H<sub>2</sub>O                                      186.1 g  
dH<sub>2</sub>O      adjust to final volume of 1L

1. Dissolve EDTA in 800 ml dH<sub>2</sub>O, adjust pH to 8.0 with NaOH (~20 g NaOH pellets)
2. Adjust to final volume of 1L and sterilize by autoclaving.

### **10 mg/ml Ethidium bromide**

Ethidium bromide 1g  
dH<sub>2</sub>O      adjust to final volume of 100 ml  
-Note: store in a dark bottle at r.t.

### **20 mg/ml Proteinase K**

1. Dissolve proteinase K at a concentration of 20 mg/ml in dH<sub>2</sub>O and dispense into aliquots and store at -20°C.

-Note: Concentration in reaction is 50 µg/ml in reaction buffer (0.01 M Tris-HCl pH 7.8, 0.005 M EDTA and 0.5% SDS) for 37~56°C.

### **RNase A**

1. Dissolve pancreatic RNase A at a concentration of 10 mg/ml in 10 mM Tris-HCl (pH 7.5) and 15 mM NaCl.
2. Heat to 100°C for 15 min, Allow to cool slowly to r.t. Dispense into aliquots and store at -20°C.

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