

2X CTAB buffer (100ml)

2.0g CTAB
10.0ml 1M Tris pH 8.0
4.0ml 0.5M EDTA pH 8.0 (EDTA will be dissolve in pH 8.0)
28.0ml 5M NaCl
40.0ml H₂O

1. Dissolve 2.0g CTAB in about 70ml of deionized water in 250ml beaker.
2. Placed the beaker on a hot plate (45°C) with a magnetic stirrer.
3. Add 10.0ml of 1M Tris pH 8.0
4. Add 0.5M EDTA to the beaker.
5. Mix the solution
6. Store at room temperature.

Adjust all to pH 5.0 with HCL and make up to 100ml with H₂O

Required reagents

2% CTAB

20mM EDTA

100mM Tris-Cl pH 8.0

1.4M NaCl

make up to 1 Litre with water, pH 7.5 - 8.0, and autoclave

+ 0.2% Mercaptoethanol

1M Tris (pH 8.0)

Dissolve 6.05 gram Tris base in 45ml of distilled water. Adjust pH to 8.0 by adding the conc HCL. Allow the solution, to cool to room temperature before making the final adjustment to the pH. Adjust the volume to 50ml. Mix with magnetic stirrer and autoclave before use.

0.5M EDTA (pH 8.0)

7.306g EDTA

50ml Distilled water

Adjust pH 8.0 by NaoH crystal and autoclave before use.

5M NaCl

14.625g NaCl

50ml Dist H₂O

Mix with magnetic stirrer

1X T.E buffer (1x Tris- EDTA)

T.E buffer is typically used for the storage of DNA

Materials

10 mM Tris-Cl, pH 8.0

1 mM EDTA

Make from 1M stock of Tris-Cl (pH 8.0) and 0.5M stock of EDTA (pH 8.0).

Procedure

1. Dissolve 0.5 ml 1M tris-Cl in about 40ml of deionized water.
2. Add 0.1ml of 0.5M EDTA.
3. Add deionized water to 50ml.
4. Store at room temperature.

10x TAE buffer (10x Tris-acetate-EDTA)

TAE buffer is typically used for agarose DNA electrophoresis

Materials

To prepare 1L of 10x solution, you need:

48.5 g Tris

11.4 mL glacial acetic acid

20 mL 0.5M EDTA (pH 8.0)

Procedure

1. Dissolve Tris in about 800 mL of deionized water.
2. Add acetic acid and EDTA.
3. Add deionized water to 1L.
4. Store at room temperature.

Dilute stock solution 10:1 to make a 1x working solution.

Reagent required in 1X TAE

40 mM Tris

20 mM acetic acid

1 mM EDTA.

make up to 1 Litre with water

10x TBE buffer (10x Tris-borate-EDTA)

TBE buffer is typically used for agarose DNA electrophoresis

Materials

To prepare 1L of 10x solution, you need:

108 g Tris base

55 g boric acid

40 ml 0.5M EDTA (pH 8.0)

Procedure

1. Dissolve Tris in about 800 mL of deionized water.
2. Add boric acid and EDTA.
3. Add deionized water to 1L.

4. Store at room temperature.

Dilute stock solution 10:1 to make a 1x working solution.

0.5x solution can also be used.

1% Agarose gel

Prepare a 1 % solution of agarose by melting 1 g of agarose in 100 mL of 1X TBE or TAE buffer in a microwave for approximately 2 min. Allow to cool for a couple of minutes.

6X Loading dye solution

10 mM Tris-HCl, pH 7.6

0.03% bromophenol blue

0.03% xylene cyanol FF

60% glycerol

60 mM EDTA

Storage:

Store at room temperature +4C upto twelve months. For longer periods, store at -20C.

Ethidium Bromide

Add 1 g of ethidium bromide to 100 ml of water. Stir on magnetic stirrer for several hours to ensure that the dye is dissolved. Wrap the container in aluminum foil or transfer the solution to dark bottle and store at room temperature (Sambrook *et al.*, 1989).

Caution:

Ethidium bromide is a powerful mutagen and is moderately toxic. Gloves should be worn when working with solution that contain this dye and mask should be worn when weighing it out. After use, these solutions should be decontaminated.

Ammonium persulfate

APS (ammonium persulfate)

5 g APS in H₂O, 50 ml final volume (10% w/v final concentration)

Make aliquots, store at -20°C

SDS-PAGE 10X gel running buffer

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248 mM Trisma (60 g)

1.92 M glycine (288 g)

1% w/v SDS (20 g)

Final volume 2 L

No need to pH, filters, or degas

Dilute to 1X for running SDS-PAGE gels