Preparation of TAE (tris, acetic acid, EDTA) and TBE (tris, boric acid, EDTA) electrophoresis buffers.

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TBE running buffer is the most commonly used buffer for DNA and RNA polyacrylamide gel electrophoresis. TBE is effective under slightly basic conditions, which keeps DNA deprotonated, water-soluble, and protected from degradation. TBE is used with non-denaturing or denaturing (7 M urea) acrylamide gels also for DNA automated sequencing gels, STR analysis and RSCA. TBE can also be used for agarose gels but is not recommended for preparative gels for recovery of nucleic acids. TAE buffer is the most commonly used buffer for DNA agarose gel electrophoresis. TAE is also used for non-denaturing RNA agarose gel electrophoresis. Double-stranded DNA runs faster in TAE than in TBE but is also exhausted much faster after extended runs. Buffer circulation or buffer replacement during extended electrophoresis can remedy the lower buffering capacity. The 1× TAE buffer is used both in the agarose gel and as a running buffer. Applied voltages of less than 5 V/cm (distance between the electrodes of the unit) are recommended for maximum resolution. Dilution of the concentrated TAE buffer produces a 1× TAE buffer with 40 mM Tris-acetate and 1 mM EDTA, pH 8.3.

Procedure

1. Prepare 100 mL of 1M HCl by adding 8.62 mL of concentrated HCl to 91 mL of dH_20 in a 250 mL flask. Do not EVER add water to the acid! Sit in magnetic stirrer for at least 5 minutes, top up to 100 mL with dH_20.

2. Prepare 100 mL of 10M NaOH by adding 40 g of NaOH to 40 mL of dH_20 in a 250 mL flask. Sit in magnetic stirrer for at least 5 minutes or until all NaOH has dissolved, top up to 100 mL with dH_20. The dissolution of NaOH in water is an exothermic reaction!

3. For TBE, prepare 0.5 M EDTA, pH 8.0 by adding 186.1 g of $Na_2EDTA\text{-}2H_2O$ in 700 ml of dH_20, adjusting pH to 8.0 (EDTA will not dissolve otherwise) by adding 10 M NaOH (approximately 50 ml), top up to 1 litre using dH_20.

4. Add all needed salts (see table below) to an appropriately sized flask.

For TAE	1 litre 10x	10 litre 10x	20 litre 10x	1 litre 50x
Tris base	48.4 g	484 g	968 g	242 g
Glacial acetic acid	11.42 ml	114.2 ml	228.4 ml	57.1 ml
$Na_2EDTA\text{-}2H_2O$	7.44 g	74.4 g	148.8 g	37.2 g
dH_2O top up to	1 l	10 l	20 l	1 l

For TBE	1 litre 10x	10 litre 10x	20 litre 10x	1 litre 50x
Tris base	108 g	1.08 kg	2.16 kg	242 g
Boric acid	55 g	550 g	1.1 kg	55 g
0.5 M EDTA, pH 8.0	40 ml	400 ml	800 ml	40 ml
dH_2O top up to	1 l	10 l	20 l	1 l

5. Add 80% of required dH_20 volume and mix on magnetic stirrer until all salts have dissolved.

6. Adjust TAE pH to 7.6 using 1M HCl or 10M NaOH (as deemed necessary) using a disposable transfer pipette, drop by drop.

7. Top up solutions with dH_20 to final recommended volume.

8. Store at room temperature.

9. To prepare 1x solution from either 10x or 50x stock, add 9 or 49 parts of dH_20, respectively.

Notes

1. To facilitate visualization of DNA fragments during the run, ethidium bromide should be added to the electrophoresis buffer (0.5 µg/ml final concentration). If buffer is prepared for both the electrophoresis tank and the gel, ensure an identical concentration of ethidium bromide.

2. High-voltage electrophoresis applications carried out over long periods of time require TBE instead of TAE as it has a greater buffering capacity.

3. DNA has faster mobility in TAE but this buffer also conducts a great amount of current causing gels to heat up.

4. Because electrophoresis buffer can accumulate nucleases, fragments intended for subsequent cloning should be isolated from gels and buffers prepared with autoclaved 50× TAE.

5. TBE buffer is generally better at producing well-defined bands on the electrophoresis gels whereas TAE results in smears.

6. TBE buffer is prone to precipitation over time, this generally will not adversely affect performance.

References

1. Current Protocols in Molecular Biology, Appendix 2, A.2.5, Supplement 40, Frederick M. Ausubel et al. 2003.

2. TBE, or not TBE; that is the question: Beneficial usage of tris-borate for obtaining a higher resolution of small DNA fragments by agarose gel electrophoresis. Yutaka Miura et al, Nagoya Medical Journal 43(1) 1-6, 1999.

Revision history

1.0 Original document.

2.0 Optimized protocol.

3.0 Changes to document format only.