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# Preparing 10x TBE Electrophoresis buffer

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TBE and TAE buffers are often used in procedures involving nucleic acids, the most common being electrophoresis. Tris-acid solutions are effective buffers for slightly basic conditions, which keep DNA deprotonated and soluble in water.

EDTA is a chelator of divalent cations, particularly of magnesium (Mg2+). As these ions are necessary cofactors for many enzymes, including contaminant nucleases, the role of the EDTA is to protect the nucleic acids against enzymatic degradation. But since Mg2+ is also a cofactor for many useful DNA-modifying enzymes such as restriction enzymes and DNA polymerases, its concentration in TBE or TAE buffers is generally kept low (typically at around 1 mM).

TBE buffer is used  to prepare agarose gels and as running buffer in agarose gel electrophoresis to separate and identify and analyse nucleic acids.

## SCOPE:

This protocol covers the steps involved in making 1L of 10x TBE buffer from Tris, Borate and EDTA powders

## DEFINITIONS:

TBE: Tris Borate EDTA

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This procedure can be performed by laboratory staff that have been trained and have theoretical and practical skills in good laboratory practices. It can also be performed by molecular biology students or students of related fields under the supervision of a laboratory staff.

**Chemicals**

- Tris
- Borate
- EDTA

**Equipment/Materials**

- Sensitive or Electronic weighing balance
- Measuring cylinder
- Distilled water
- Magnetic stirrer
- 500ml beaker

## Preparing 10x TBE buffer solution (1 liter) 40m

1 Use an electronic balance to weigh out **108 g** Tris base (CAS# 77-86-1, free base) into a **1000 mL** ml or higher volume beaker ( depending on what volume of beaker is available and should be big enough to contain the powders to be dissolved). 40m

2 Weigh **55 g** boric acid (CAS# 10043-35-3) into the same beaker

3 Weigh **9.3 g** EDTA, disodium salt dihydrate (Cas# 6381-92-6, $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, MW: 372.24) into a weighing boat and pour into the beaker.

Note that you can also use **[M]0.5 Molarity (M)** EDTA solution at **pH8.3**. In this case skip to Step 4. Then add **40 mL** EDTA solution to the dissolved salts.



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4 Measure **100 mL** distilled or deionised water using a measuring cylinder, pour into the **1000 mL** beaker and stir for **00:01:00** either manually or using a magnetic stirrer and flea. 1m

If the TBE buffer will be used for RNA work, ensure that RNase-free water is used.

5 Adjust volume to **1 L** by adding **900 mL** of distilled or deionised water.

6 Place the beaker on top of a magnetic stirrer and put a magnetic flea in the solution. Allow to stir until you have a clear solution, this may take up to **00:30:00** 30m

7 Check the pH using a pH meter or Universal indicator strips and record the result for quality control. A properly prepared solution should be pH **8.3** and should not require adjustment.

8 Autoclave (if sterile solution is needed) and pour solution into clean, autoclaved and labelled glass bottles.

10 x TBE buffer is stable for up to 6 months at room temperature. The solution should be discarded if it becomes cloudy.

## Make 1 x TBE working solution

9 This buffer can be diluted to 1x each time before use by diluting **100 mL** of 10x TBE with **900 mL** of distilled water to have **1 L** of 1x TBE buffer.



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