

## Buffers

### TAE buffer series

40 mM trisaminomethane (Tris), 20 mM acetic acid, 1 mM ethylenediaminetetraacetic acid (EDTA) disodium salt (pH ~8). We typically add 12.5 mM magnesium acetate in the solution for application related to DNA (termed TAEM buffer).

- 1 L 50× TA buffer (we have > 0.9 L left in the lab in April 2022). Weight 242 g of Tris and 57.1 mL of acetic acid and dissolve in 0.7 L of DI water. Add water to final volume of 1 L
- 50 mL 10× TAE buffer. Weight 0.186 g EDTA disodium salt and add to 10 mL 50× TA buffer. Add DI water to final volume of 50 mL. Notes:
  - EDTA dissolves really slow. When preparing, planning ahead is very important
  - Sometimes we need 2× of regular EDTA concentration in the final regular buffer. In such a case, weight 0.372 g EDTA disodium salt and the rest are the same
  - Normally, the concentration is marked as '10× TAE buffer. For 1×, 40 20 1 or 40 20 2'. The numbers are concentration of 3 components in mM
  - When we need '10× TAE buffer. For 1×, 40 20 1' with '10× TAE buffer. For 1×, 40 20 2' and '10× TAE buffer. For 1×, 40 20 0' available, we can mix these 2 under 1:1 volume ratio and create '10× TAE buffer. For 1×, 40 20 1'
- 10 mL 1× TAEM buffer. Add 5 – 8 mL DI water first, then add 1 mL 10× TAE buffer and 125 µL of 1 M magnesium acetate. Add water to final volume of 10 mL. Notes:
  - Sometimes we need  $\text{Mg}^{2+}$  concentration to be 6 mM instead of 12.5 mM. Therefore, 60 µL of 1 M magnesium acetate should be added
  - Similar to Note c in step 2, we can mix TAEM buffer with 6 and 12.5 mM  $\text{Mg}^{2+}$  to create TAEM buffer with  $\text{Mg}^{2+}$  between 6 and 12.5 mM with a suitable volume ratio
- 7.5 mL 4/3× TAEM buffer. Add 5 – 6 mL DI water first, then add 1 mL 10× TAE buffer and 125 µL of 1 M magnesium acetate. Add water to final volume of 7.5 mL. The same notes in step 3 apply here
- 1.2 mL fixing buffer (TAEM buffer with 2 mM nickel chloride). Add 900 µL of 4/3× TAEM buffer and 96 µL of 25 mM nickel chloride solution (we have quite some in stock)
  - We can start with different concentrated TAEM buffer and nickel chloride solution. Just make sure the final concentration is desired
  - We may have different combination of EDTA,  $\text{Mg}^{2+}$  and  $\text{Ni}^{2+}$  concentration. The most important aspect is the correct and clear marking. Without it, the buffers are useless

### MES buffer

50 mM 2-(N-morpholino)ethanesulfonic acid (MES), 5 mM magnesium chloride, and 200 mM sodium chloride (pH ~6.5)

- 50 mL 0.5 M MES sodium salt solution. Weight 5.43 g MES sodium salt and dissolve in 40 mL of DI water. Add water to final volume of 50 mL
- 10 mL 2 M sodium chloride solution. Weight 1.169 g sodium chloride and dissolve in 7 mL of DI water. Add water to final volume of 10 mL

3. 50 mL 2 M magnesium chloride solution. Weight 20.33 g magnesium chloride hexahydrate. Add DI water to final volume of 50 mL. Notes:
  - a. Magnesium chloride hexahydrate can get really messy due to the water in the salt. Weighting has to be quick otherwise the crystals will dry
  - b. If magnesium chloride is not available, magnesium acetate can be used in place of it. Don't dilute 1 M magnesium acetate unless in the buffer. For example, don't make 50 mM magnesium acetate in DI water alone. Rather, make 10× MES buffer where there are 50 mM magnesium acetate
4. 50 mL 1× MES buffer. Add 35 mL DI water first, then add 5 mL of 0.5 M MES sodium salt, 125 µL of 2 M magnesium chloride, and 5 mL of 2 M sodium chloride. Add water to final volume of 50 mL
5. Sometimes we need 1× MES buffer with nickel chloride. Similar to step 5 in making TAEM buffer, adding nickel chloride to concentrated MES buffer followed by dilution will be all we need. Typical final  $\text{Ni}^{2+}$  concentration is 2 – 3 mM

### **TBE buffer series**

89 mM trisaminomethane (Tris), 89 mM boric acid, 2 mM ethylenediaminetetraacetic acid (EDTA) disodium salt (pH ~8.3). Normally purchased directly from Thermo Fisher in 10× concentration. There is a problem with precipitation due to the high concentration. Currently we just use the 10× buffer with precipitations at the bottom and pretend the precipitations don't exist. Dr Chengde Mao's student, Dake Mao, suggested dilution into 5× concentration. We typically use 0.5× TBE buffer with 11 mM magnesium chloride for application related to agarose gel of DNA.

0.5 L 0.5× TBE with 11 mM magnesium. Add 0.4 L DI water first, then add 25 mL 10× TBE buffer and 2.75 mL of 2 M magnesium chloride. Add water to final volume of 0.5 L.

### **TRIS Buffer**

#### **1. 10X Sodium Tris-HCl/EDTA Buffer**

Dissolve 24.2 g of Tris base, 58.5 g of NaCl and 1.9 g of EDTA in 900 mL DI water. Give a vigorous mix till all the salts are dissolved in the solution. Add HCl (~13 mL) till the solution reaches a pH value of 7.4. Add water to make the total volume of solution to 1 L.

#### **2. 1X Tris**

Mix 1:9 volume ratio of 10X Tris: DI Water. Add additional NaCl (59 mg/L).