Buffers

TAE buffer series

40 mM trisaminomethane (Tris), 20 mM acetic acid, 1 mM ethylenedia-minetetraacetic 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. 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
2. 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:
    a. EDTA dissolves really slow. When preparing, planning ahead is very important
    b. 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
    c. 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
    d. 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'
3. 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:
    a. Sometimes we need Mg^{2+} concentration to be 6 mM instead of 12.5 mM. Therefore, 60 µL of 1 M magnesium acetate should be added
    b. Similar to Note c in step 2, we can mix TAEM buffer with 6 and 12.5 mM Mg^{2+} to create TAEM buffer with Mg^{2+} between 6 and 12.5 mM with a suitable volume ratio
4. 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
5. 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)
    a. We can start with different concentrated TAEM buffer and nickel chloride solution. Just make sure the final concentration is desired
    b. We may have different combination of EDTA, Mg^{2+} and 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)

1. 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
2. 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 Ni^{2+} concentration is 2 – 3 mM

TBE buffer series

89 mM trisaminomethane (Tris), 89 mM boric acid, 2 mM ethylenedia-minetetraacetic 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.