NPPC protocol on Buffer preparation

Remember to use a measuring cylinder to calibrate your flasks/bottles. Example; measure 500ml or 1000ml water with the measuring cylinder and pour to the empty flask that you are going to use for the final volume of 500ml/1 litre flask. Mark on the flask or the bottle where the volume has reached. This marking will help you to adjust your final volume whether it is 500ml or 1000ml after dissolving all solutes and other additives like HCL or NaOH.

1M Tris HCL for 500ml volume (Molecular weight of Tris = 121.1g/M)

1. Add 60.55 g of Tris in a 500ml or 1 litre flask
2. Then add 400ml of distilled water
3. Dissolve using magnetic stirrer
4. Measure pH with pH meter and adjust to pH 8 by adding HCL drop by drop and stir
5. Adjust final volume to 500ml
6. (if you add 121.1 g of Tris in 1L = 1M, so half amount for half volume)

Tris HCL*: Tris hydrochloride, is a buffering agent (acidic buffer) commonly used by molecular biologists to adjust the pH of a solution or stabilize the pH.*

0.5M of EDTA (MW = 372.24g/mol) pH 8 for 500ml

Here the concentration is 0.5M. if you add half amount of the molecular weight that is 186.12g in 500ml then you will get 1M. which means to achieve a 0.5M in 1L, you will need to add half the amount (372.24 divided by 2 = 185.12g). For 500ml of 0.5M, you will need to add half of 186.12g which is 93.06g.

1. Add 93.06g in a 1L flask
2. Add 400ml of distilled water and stir using magnetic stirrer. Add pellets/powder of NaOH to dissolve EDTA which otherwise will remain very compact. Add NaOH in smaller amount.
3. Once EDTA has dissolved (i.e. becomes a clear liquid), adjust pH by HCL to pH 8.
4. Adjust final volume to 500ml

Autoclave both Tris HCL and EDTA at 120°C for 15 minutes.

****EDTA:*** *Ethylenediamine tetra acetic acid, is a chelating agent that binds divalent metal ions such as calcium and magnesium. EDTA can be used to prevent degradation of DNA and RNA and to inactivate nucleases that require metal ions. EDTA can also be used to inactivate metal ion-requiring enzymes.*

50ml of 10X TE buffer

1. Take 44ml of sterile distilled water in a 15oml or 250 ml bottle (use sterilized bottles)
2. Add 5ml of 1M Tris HCl, and 1ml of 0.5M EDTA (use autoclaved ones)
3. Mix well
   It is better to add Tris HLC and EDTA to water

10ml of 1X TE buffer

1. Add 9ml of sterile distilled water
2. Add 1ml of 10X TE buffer
3. Mix well

TE: *Tris-EDTA, are use in maintaining the pH of the solution and solubilizing DNA or RNA while protecting the nucleic acids from enzymatic lysis. TE buffer is made up of Tris, a pH buffer and EDTA, a metal chelating ion. It is used in DNA extraction processes to lyse, wash and dissolve DNA. TE buffer is also used for DNA storage because of its ability to store DNA for a longer period of time without degrading it.*

CTAB (N-Cetyl-N, N, N-Trimethylammonium Bromide) for 1 litre volume

Warm some sterile distilled water I the water bath or on hot plate before preparing this.

1. Weigh and add 20g of CTAB and put in a 1 litre flask
2. Then add 82g of NaCl and 20g of PVP 10,000. (If the PVP is 40,000 or PV40, then the amount should be four times less.
3. Add 400ml of warm sterile distilled water (~50°C) (CTAB dissolves better in warm water)
4. Add 100ml of 1M Tris HCL (use the autoclaved one)
5. Add 40ml of 0.5M EDTA (use the autoclaved one)
6. Dissolve CTAB on hot plate at 50°C and stirring with a magnetic stirrer. (This takes time, it is better to prepared in the morning or late afternoon and keep CTAB stirring overnight if it takes too long to dissolve
7. Adjust final volume to 1 litre. **(Do not autoclave CTAB)**
8. Do not add β-mercapto to the stock of CTAB

PVP*: Polyvinylpyrrolidone,*

Chloroform / Isoamyl alcohol (24:1)

Add 1ml of isoamylic alcohol to 24ml of chloroform. Prepare bulk of 250ml by adding 10ml isoamylic alcohol to 240ml chloroform.

Ethanol 70% for final volume of 200ml (use molecular grade for extraction).

Use the other ethanol for cleaning purposes.

140ml ethanol (100% or 99%) plus 60ml sterilized distilled water.

Notes

1. Prepare fresh Tris-HCL and EDTA every six months. CTAB should be prepared one to two days before extraction and add β-mercapto just before dispensing into the extraction tubes or products.
2. Sterilize all glass wares used for CTAB and TE buffer.