

"BIG VOLUME" SOLUTIONS

Solutions should be prepared with distilled and autoclaved water unless otherwise indicated. If the solutions have to be left unused for long periods (> 1 week), it is recommended to store them in fridges or freezers unless otherwise indicated. Acquire "molecular biology grade" reagents in all cases.

20xSSC

For 1 litre: 175.3 g NaCl

88.2 g Na₃C₆H₅O₇·2H₂O (sodium citrate)

Adjust to pH=7 with some HCl if too basic, or NaOH if too acid (*but it will be quite close to neutrality even without adding any of these*)

Make up to 1 litre with dH₂O.

(It may take time, up to some hours, to dissolve the salts, so it is better to prepare it well in advance of when it is needed)

2xSSC For 1 litre: 100 ml 20xSSC + 900 ml dH₂O

4xSSC For 1 litre: 200 ml 20xSSC + 800 ml dH₂O

0.1xSSC For 1 litre: 5 ml 20xSSC + 995 ml dH₂O

4xSSCT (4xSSC+0.1% Tween 20) For 1 litre: 200 ml 20xSSC + 800 ml dH₂O + 10 ml Tween 20 (*do not autoclave*)

SSC buffers function as (high) ionic strength buffers to facilitate hybridisation of probes to specific nucleic acid sequences. *These substances are not considered as hazardous.*



10xPBS

For 1 litre: 75.52 g NaCl (1.3M)
22.07 g Na₂HPO₄·12H₂O (0.07M) (or 12.46 g Na₂HPO₄·2H₂O)
4.68 g NaHPO₄·2H₂O (0.03M)
Adjust to pH=7.4 as explained for 20xSSC.
Make up to 1 litre with dH₂O.

Keep at RT

1xPBS For 1 litre: 100 ml 10xPBS + 900 ml dH₂O

These substances are not considered as hazardous.

1N HCl

For 1 litre: 83.26 ml HCl 37%, make up to 1 litre with dH₂O. Store at room temperature, light protected. Flammable and corrosive. Do not autoclave!

0.1N HCl For 1 litre: 100 ml 1N HCl + 900 ml dH₂O

0.01N HCl For 500 ml: 5 ml 1N HCl + 495 ml dH₂O

Although these concentrations are low, HCl is irritant and corrosive and can cause important skin burns and eye damage if in contact. Work with gloves, glasses, clothes and protection mask.

1N NaOH

For 1 litre: 40 g NaOH, add 1 litre dH₂O. Store at room temperature and in darkness. Do not autoclave!

Although the concentration is low, the NaOH powder can cause serious skin burns and eye damage if in contact. Work with gloves, glasses, clothes and protection mask.

100% EtOH (prepare one 500 ml bottle and store it in the freezer. Do not autoclave!)

90% EtOH (prepare one 500 ml bottle and store it in the freezer. Do not autoclave!)

70% EtOH (prepare one 500 ml bottle and store it in the freezer. Do not autoclave!)

50% EtOH (prepare one 500 ml bottle and store it in the freezer. Do not autoclave!)

EtOH is flammable.

Extemporaneous acetic acid 45-60% solutions (with glacial acetic acid and dH₂O)



0.05M Citrate buffer (pH=4.8)

0.05M citric acid ($C_6H_8O_7 \cdot H_2O$, 10.51 g/l) in dH₂O

0.05M sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, 14.71 g/l) in dH₂O

For pH=4.8 it requires about 667 ml of 0.05M citric acid solution and 1 litre of 0.05M sodium citrate solution.

0.01M Citrate buffer (pH=4.8) For 500 ml: 100 ml 0.05 M Citrate buffer + 400 ml dH₂O

Citrate buffer can cause eye irritation. Work with appropriate protection equipment.

1x "enzyme buffer"

0.1 M citric acid ($C_6H_8O_7 \cdot H_2O$, 21.02 g/l) in dH₂O (solution A)

0.1 M trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, 29.42 g/l) in dH₂O (solution B)

Mix 6 volumes of solution A and 4 volumes solution B. Dilute 1:10.

Enzymatic solutions

Enzyme mix "Dr. Siljak-Yakovlev"

4% Cellulase R10

1% Pectolyase Y23

4% Hemicellulase Sigma

Dissolve in 0.05M citrate buffer (pH=4.8)

For 10 ml: 0.4 g cellulase + 0.1 g pectolyase + 0.4 g hemicellulase

Enzyme mix "Dr. Siroky"

0.4% Pectolyase Sigma (or Macerozyme R10 from Duchefa # M8002 0010)

0.4% Cytohelicase Sigma (Sigma # C-8274)

0.4 % Cellulase Onozuka Serva (or Cellulase Onozuka RS, Duchefa # C8003 0010)

Dissolve in 1x "enzyme buffer"

For 100 ml: 0.4 g pectolyase + 0.4 g cytohelicase + 0.4 g cellulase



“SMALL-MEDIUM VOLUME” SOLUTIONS

Solutions should be prepared with autoclaved and distilled water unless otherwise indicated. It is recommended to store them in aliquots (in regular microcentrifuge tubes) at -20°C, unless otherwise indicated. Acquire “molecular biology grade” reagents in all cases.

RNAse A 100 µg/ml

RNAse A (stock solution 100 mg/ml), dilute in 2xSSC to a final concentration of 100 µg/ml (1 µl stock solution + 999 µl 2xSSC)

Ribonuclease A is a chromatographically purified, pyrimidine-specific endoribonuclease that acts on single-stranded RNA (eliminating most of it). Non-hazardous, but possible sensitisation.

Pepsin 0.05 mg/ml

Pepsin (stock solution 10 mg/ml), dilute 1:200 in 0.01N HCl (10 µl stock solution + 2 ml 0.01 HCl – aliquot in two 1.50 Epp. tubes).

Pepsin is a peptidase used for the proteolytic digestion. Eye, skin and respiratory tract irritant. Possible sensitisation by inhalation. Wear gloves and mask.

Neutral 3.7% formaldehyde (extemporaneous)*

Formaldehyde for microscopy, free from acid (37%), dilute 1:10 in 1xPBS (for 110 ml: 10 ml formaldehyde + 100 ml 1xPBS)

*Formaldehyde is useful as a preservative and stabiliser, particularly to preserve chromosome morphology in *in situ* experiments. Very toxic reagent with danger of irreversible effects through inhalation, skin contact or ingestion. It causes burns and possible carcinogens effects. It may cause sensitisation by skin contact. Always work under the hood and protected appropriately (gloves, mask and protection clothes).*

(*) Non-essential reagents for our regular protocol.



50% Dextran sulphate

It can be acquired commercially in this form. However, if you have the DS powder then dilute 50 g of dextran sulphate in 100 ml of dH₂O. It may not be easy to solve, so you can try either to warm it to 65°C to get into solution, or let sit overnight at room temperature, or stir overnight at 4 °C. It should be warmed at least at RT prior to use in dilution for best results. Do not vortex before using as air bubbles may be formed the removal of which takes time.

Dextran sulphate is used as an additive in probe hybridisation solutions. Its increases the rate of hybridisation by 10 fold, favouring the formation of probe networks. This substance is not considered as hazardous.

Proteinase K 1 mg/ml*

Dissolve 1 mg in 1 ml dH₂O (stock solution). Before use: dilute 100x in 2xSSC.

Proteinase K exhibits strong proteolytic activity on a wide variety of denatured and native proteins, and endogenous nucleases, of high molecular weight. Eye, skin and respiratory tract irritant. Possible sensitisation by inhalation. Wear gloves and mask.

Deionised formamide

Use it directly, undiluted, as provided by the company. Store at room temperature, protected from light.

Formamide takes part on hybridisation mixture solutions (containing the probes) to increase probe hybridisation specificity. By disrupting hydrogen bonds, it denatures nucleic acids. In DNA to DNA hybridisation, each 1% increase in formamide concentration lowers the T_m by 0.7%. This is a very toxic and dangerous reagent. Use breathing protection (mask) and protection glasses. Work under the hood. Avoid breathing its vapours, mist or gas. Avoid eye, skin and clothes contact. Always use gloves and wash your hands after manipulating the substance. Work in a place with adequate ventilation. Although it is not a potential carcinogen, it can cause foetal malformations and be noxious for the human reproductive system.

Sodium dodecyl sulphate (SDS) 0.1 g/ml*

Dissolve 1 g in 10 ml sterile dH₂O.

Anionic detergent. It can be incorporated in the hybridisation mixture at 0.6% concentration. It is noxious in case of ingestion, toxic if any skin contact. It can irritate skin and respiratory tract and provoke eye damage. Avoid powder inhalation. Always wear protection gloves, glasses and mask.

(*) Non-essential reagents for our regular protocol.



Mounting medium

We regularly use “Vectashield Mounting Medium with DAPI” (Vector Laboratories), stored at 4 °C in dark conditions.

This mounting medium inhibits photobleaching of most fluorochromes, also after prolonged storage of mounted slides. DAPI produces a blue fluorescence when bound to DNA (with excitation at about 360 nm and emission at 460 nm). Harmful by inhalation, in contact with skin and if swallowed. Irritating to eyes, respiratory system and skin.

