Appendix I

Solutions

Solutions used in the protocols described in chapter 2 and in appendix II are presented in alphabetic order in the present appendix. Solutions required for the preparation of working reagents are also described herein.

Alcian blue 8 GX solution

Preparation of 100ml of solution:

20mg alcian blue 8 GX (C.I. 74240, Sigma-Aldrich)

70ml ethanol (Merck)

30ml glacial acetic acid (Merck)

Mix all the reagents and let the dye dissolve completely. Store almost indefinitely at 4ºC in the dark.

Alizarin red S 1% - stock solution

Preparation of 100ml of stock solution:

1g alizarin red S (C.I. 58005 Sigma-Aldrich)

Dissolve in 100ml of 1% potassium hydroxide solution (see below).

Alizarin red S - working solution

Preparation of 100ml of working solution:

Dilute 250µl of the stock solution of alizarin red S in 100ml of 1% potassium hydroxide solution (see below). Store at room temperature.

Aqueous toluidine blue solution 1% pH 4.5

Preparation of 100ml of solution:

1g toluidine blue (C.I. 52040, Sigma-Aldrich)

Dissolve the toluidine blue in 100ml of distilled water and adjust the pH to 4.5 with concentrated acetic acid. Store at room temperature.

Blocking solution 2% or 1%

Preparation of 100ml of blocking solution:

2g or 1g blocking reagent (Boehringer Mannheim GmbH; for 2% and 1% solution, respectively)

Dissolve in 100ml of maleic acid buffer (MAB, see below) at 65ºC mixing occasionally. Cool to room temperature, aliquot and store at -20ºC.

Bouin-Holland solution – stock solution

Preparation of 100ml of stock solution:

4g picric acid

2.5g copper acetate

Dissolve in 100ml of distilled water and store in a dark bottle, at room temperature.

Bouin-Holland solution – working solution

To 100ml of Bouin-Holland stock solution add 10 ml of 37% paraformaldehyde solution (Sigma-Aldrich) and 10ml of a saturated solution of mercury chloride, mix well and use immediately.

Bovine serum albumin solution (BSA, 10mg.ml^{-1})

Preparation of 10ml of solution:

100mg of bovine serum albumin (Randox, Ireland)

Dissolve in 10ml of sterile water. Aliquot and store at -20ºC.

CHAPS 2% (3-[3-cholamidpropyl)dimethylammonio]-1-propanesulfonate)

Preparation of 100ml of solution:

2g CHAPS (Sigma-Aldrich)

Dissolve in 100ml of diethyl pyrocarbonate treated, sterile water (see below)

and store at room temperature.

Citric Acid 2M

Preparation of 100ml of solution:

42g citric acid (Merck)

Dissolve in 100ml of MilliQ water. Autoclave for 20 minutes at 121^{o}C and store at room temperature.

50×Denhardt's solution

Preparation of 100ml of solution:

1g bovine serum albumin

1g ficoll (Sigma-Aldrich)

1g polyvinilpirrolidone (PVP, Sigma-Aldrich)

Dissolve overnight at 4^{o}C, filter, aliquot and store at -20ºC.

DEPC treated Ethylenediaminetetraacetic acid (EDTA, 0.2M and 0.5M)

Preparation of 100ml solution:

7.6g (0.2M) EDTA (Sigma-Aldrich)

19g (0.5M) EDTA

Dissolve the appropriate quantity of EDTA for the molarity desired, in DEPC water (see below) by adjusting the pH to 8 with sodium hydroxide and mixing well. Adjust the final volume of the solution to 100ml with DEPC water. Store at room temperature.

Developing buffer (100mM TRIS-HCl, 100mM NaCl, 50mM $MgCl_2$, pH 9.5)

Preparation of 100ml of solution:

10ml of 1M tris-HCl solution, pH 9.5 (see below)

2ml of 5M sodium chloride solution (see below)

5ml of 1M magnesium chloride solution (see below)

Mix together appropriate volumes of the solution and make up to 100ml with double distilled water and use immediately.

Diethyl pyrocarbonate treated water (DEPC water)

Preparation of 100ml of solution:

Fill a 100ml Duran bottle with MilliQ water and add 10µL of DEPC (diethyl pyrocarbonate, Sigma-Aldrich). Mix vigorously and leave to stand at room temperature for at least 1 hour before autoclaving for 20 minutes at 121^{o}C. Store at room temperature.

Eosin Y 1% aqueous solution

Preparation of 100ml solution:

1g eosin Y (C.I. 45380, Sigma-Aldrich)

Dissolve the eosin in 100ml of double distilled water and store until use.

Harris haematoxylin solution

Preparation of 100ml solution:

1g haematoxylin (C.I.75290, Merck)

10g aluminium potassium sulphate.12 H_2O (Merck)

0.25g mercuric oxide (Merck)

4ml glacial acetic acid

5ml absolute ethanol

Dissolve the haematoxylin in 5ml of absolute ethanol. Dissolve the aluminium potassium sulphate in 100ml of warmed distilled water. Combine the solutions and boil for 4 minutes, remove from heat and add the mercuric oxide, mix well and then boil for 1 minute or until the dye becomes a dark purple colour. Cool the solution rapidly, under running water and add 4ml of glacial acetic acid and filter. Immediately prior to use dilute 50:50 in absolute ethanol and filter the

resulting solution. Store the stock solution in the dark at room temperature.

Heparin (10mg.ml^{-1})

Preparation of 10ml solution:

100mg heparin (Sigma-Aldrich)

Dissolve the heparin in 10ml of MilliQ water, aliquot and store at -20ºC.

Hybridization buffer, for Northern blot

Preparation of 100ml solution:

25ml 20×SSC (see below)

10ml 50×Denhardt's solution (see above)

1ml sodium dodecyl sulphate solution 10% (SDS, see below)

100µl torula RNA yeast solution (50mg.ml^{-1}, see below)

Mix all the reagents and make up the resulting solution to a final volume of 100ml with sterile DEPC water (see above). Store at -20ºC.

***In situ* hybridization solution**

Preparation of 100ml solution:

50ml deionized formamide (Sigma-Aldrich)

20ml sterile 20×SSC Buffer (see below)

2ml 50×Denhardt's solution (see above)

2ml torula RNA yeast (50mg.ml^{-1}, see below)

2ml 2% CHAPS (see above)

1ml heparin (10 mg.ml^{-1}, see above)

Mix all the reagents together and adjust the pH to 6.0 with 2M citric acid solution (see above). Make up the resulting solution to a final volume of 100ml with sterile DEPC water (see above). Aliquot and store at -20ºC.

Light green dye solution

Preparation of 100ml solution:

0.2g citric acid (Sigma-Aldrich)

10ml distilled water

0.2g light green (C.I. 42095, Sigma-Aldrich)

Prepare a solution of 2% citric acid by dissolving it in the distilled water (10ml). To this solution add the light green and mix well to dissolve. Store the stock solution in the dark at room temperature. To obtain a working solution of 2% light green, just before use, dilute 1:10 with distilled water.

Lithium chloride 4M (DEPC treated)

Preparation of 100ml solution:

16.96g lithium chloride (Sigma-Aldrich)

Dissolve in 100ml of DEPC water (see above) and store at room temperature.

Magnesium chloride solution 1M

Preparation of 100ml solution:

20.33g magnesium chloride, hexahydrate (Sigma-Aldrich)

Dissolve in 100ml of double distilled water and store at room temperature.

Maleic acid buffer (MAB; 100mM maleic acid, 150mM NaCl, 0.1% tween-20, pH 7.5)

Preparation of 100ml solution:

1.161g maleic acid (Sigma-Aldrich)

0.8775g sodium chloride (Sigma-Aldrich)

Dissolve in MilliQ water and adjust the pH to 7.5. Complete the volume to 100ml and autoclave for 20 minutes at 121ºC. Allow to cool to room temperature and add 100µl of tween-20. Store at room temperature.

Paraformaldehyde 4%, for *in situ* hybridization

Preparation of 100ml solution:

Dilute 10.8ml of 37% paraformaldehyde solution (Sigma-Aldrich) with 89.2ml of phosphate-tween buffer (PTW, see below) and use immediately.

Paraformaldehyde 4% (PFA, pH 7.4), for tissue fixation

Preparation of 100ml solution:

4g paraformaldehyde (Sigma-Aldrich)

90ml of sterile MilliQ water

10µL sodium hydroxide (saturated solution)

Mix the paraformaldehyde in 90ml of water and heat to 65ºC stirring continuously in a fume cupboard until the paraformaldehyde is completely dissolved. Allow the solution to cool to room temperature and then add 10ml of sterile 1M Phosphate Buffer pH 7.4 (PBS, see below) and with the sodium hydroxide correct the pH to 7.4 if necessary. The solution of fixative can be stored for 1 week at 4ºC.

Phosphate-carragenin-triton buffer (PCT buffer)

Preparation of 100ml solution:

50ml 1×PBS (see below)

0.7g carragenin (Sigma)

500µl triton-X (Sigma-Aldrich)

Mix all the reagents and leave them to dissolve overnight at 4^{o}C with gentle agitation. Make the volume of the solution up to 100ml with 1×PBS and store at 4^{o}C.

Phosphate buffer 1M pH 7.4 (PB), for the preparation of PFA solutions

Preparation of 100ml solution:

12.46g disodium hydrogen phosphate, dihydrate ($Na_2HPO_4.2H_2O$, Merck)

4.68g sodium dihydrogen phosphate, dihydrate ($NaH_2PO_4.2H_2O$, Merck)

Dissolve the reagents in 60ml of MilliQ water and adjust the pH to 7.4 and make the solution up to a final volume of 100ml. Autoclave the solution for 20 minutes at 121^oC. Store at room temperature.

10×Phosphate buffered saline pH 7.0 (PBS), for ISH and IHC

To prepare 100ml:

7.597g sodium chloride

1.246g disodium hydrogen phosphate, dihydrate ($Na_2HPO_4.2H_2O$)

0.48g sodium dihydrogen phosphate, dihydrate ($NaH_2PO_4.2H_2O$)

Dissolve the reagents in 60ml of MilliQ water, adjust the pH to 7.0 and make the solution up to a final volume of 100ml. Autoclave the solution for 20 minutes at 121^oC. Store the stock at room temperature.

To prepare 100ml of **1×PBS Buffer** dilute 10ml of 10×PBS buffer with 90ml of DEPC water (see above).

Phosphate-triton buffer (PBST)

Preparation of 100ml solution:

To 100ml of 1×PBS (see above) add 100µL of triton-X (Sigma-Aldrich) and mix well to completely dissolve the triton-X.

Phosphate-tween buffer (PTW)

Preparation of 100ml solution:

To 100ml of 1×PBS (see above) add 100µL of tween-20 and mix well to completely dissolve the tween-20.

Phosphomolybdic acid 1%

Preparation of 100ml solution:

1g phosphomolybdic acid (Merck)

Dissolve the phosphomolybdic acid in 100ml of distilled water. Store at room temperature.

Potassium hydroxide solution 1% (KOH)

Preparation of 100ml solution:

1g potassium hydroxide (pellets, Merck)

Dissolve the potassium hydroxide in 100ml of distilled water. Store at room temperature.

Proteinase K – stock solution (1mg.ml^{-1})

Preparation of 10ml solution:

10mg proteinase K (Sigma-Aldrich, >30 U/mg)

Dilute the proteinase K in 10ml of sterile water. Aliquot and store at -20ºC.

The working solution (0.01mg.ml-1) is prepared by diluting an appropriate volume of the stock solution with PTW (see above) and used immediately.

Sodium acetate 3M, pH 5.2

Preparation of 100ml solution:

24.6g sodium acetate (Merck)

Dissolve the sodium acetate in 60ml of distilled water and adjust the pH to 5.2 with acetic acid. Make the final volume of the solution up to 100ml with distilled water. Store at room temperature.

Sodium chloride solution 5M

Preparation of 100ml solution:

29.25g sodium chloride

Dissolve the sodium chloride in 100ml of double distilled water. Store at room

temperature.

20×sodium chloride-sodium citrate buffer (20×SSC)

Preparation of 100ml solution:

17.53g sodium chloride

8.82g sodium citrate

Dissolve all the reagents in 60ml of distilled water. Adjust the pH to 7.0 with acetic acid and complete the volume to 100ml. Autoclave the solution for 20 minutes at 121ºC. Store the stock solution at room temperature.

To prepare 100ml of **2×SSC** dilute 10ml of 20×SSC with 90ml of sterile water. All other concentrations of SSC buffer should be prepared as described using the appropriate amount of concentrated solution.

Sodium dodecyl sulphate 10% (SDS)

Preparation of 100ml solution:

10g sodium dodecyl sulphate (SDS, Merck)

Dissolve in distilled water without agitation to avoid bubble formation. Store at room temperature.

Torula RNA yeast (50mg.ml^{-1})

Preparation of 10ml solution:

0.5g torula RNA yeast powder (Roche diagnostics)

Add 10ml of distilled water to the RNA yeast powder and 50µl of saturated sodium hydroxide and warm the solution up to approximately 60ºC until it is completely dissolved. Allow the solution to cool, prepare aliquots and store at -20ºC.

10×Tris-borate-EDTA buffer (10×TBE)

Preparation of 100ml solution:

10.8g tris base (Sigma-Aldrich)

5.5g boric acid (Sigma-Aldrich)

10ml EDTA 0.5M pH 8.0 (see above)

Dissolve the salts in double distilled water and make the volume up to 100ml. Autoclave the solution for 20 minutes at 121^{o}C. Store the stock solution at room temperature.

To prepare 100ml of **1×TBE Buffer** dilute 10ml of 10×TBE Buffer with 90ml of double distilled water (for gels to run DNA samples) or DEPC water (for gels to run RNA samples).

1×Tris buffered saline (1×TBS) pH 7.4

Preparation of 100ml solution:

1.2114g tris (tris(hydroxymethyl)aminomethane, Merck)

0.8775g sodium chloride

Dissolve the salts in MilliQ water and adjust the pH to 7.4 with concentrated hydrochloric acid (HCl) and make the volume up to 100ml. Autoclave the solution for 20 minutes at 121^{o}C. Store at room temperature.

1M Tris-HCl solution, pH 7.5 (or pH 9.5)

Preparation of 100ml solution:

12.114g tris (Tris(hydroxymethyl)aminomethane)

Dissolve the tris in 60ml of double distilled water and adjust the pH to 7.5 or 9.5 with concentrated hydrochloric acid or sodium hydroxide respectively. Make the volume of the solution up to 100ml with distilled water. Store at room temperature.

Tris-NaCl solution (100 mM TRIS-HCl, 150 mM NaCl, pH 7.5)

Preparation of 100ml solution:

10ml of 1M tris-HCl solution, pH 7.5 (see above)

3ml of 5M sodium chloride solution (see above)

Mix both solutions and complete the volume to 100ml with double distilled water and use immediately.

van Gieson solution

Preparation of 100ml solution:

0.0535g acid fuchsin (C.I.42865, Sigma-Aldrich)

46.4ml distilled water

53.6ml saturated solution of picric acid (Merck)

Dissolve the acid fuchsin in 10.7ml of distilled water to obtain a 0.5% aqueous acid fuchsin solution. Add this solution to 53.6ml of the saturated picric acid and 35.7ml of distilled water to give a final volume of 100ml of van Gieson solution. To the working solution add 18µL of concentrated hydrochloric acid to sharpen colour differentiation. Store in the dark at room temperature.

Xylidine ponceau solution

Preparation of 100ml solution:

0.25g xylidine ponceau 2R (C.I. 16150, Sigma-Aldrich)

0.25g acid fuchsin (C.I. 42685, Sigma-Aldrich)

100ml 1% acetic acid (1ml Glacial acetic acid + 99ml Distilled water)

Dissolve the xylidine ponceau 2R in 50ml of 1 % acetic acid. Dissolve the acid fuchsin in 50ml of 1 % acetic acid. Store the stock solutions in the dark at room temperature. Just before use mix the two solutions to obtain a working solution of the stain.