Slide Subbing Solution

Warm 500 mL of dH2O to 50-60°C

Once at temperature, turn off heat and add,

1.25 g Gelatin Type A

0.125 g Chromium Potassium Sulfate

Cryoprotectant Solution (4000 mL)

2000 mL of pre-made PBS stock solution (pH 7.2)

(or 2000 mL dH2O + 2.8 g NaH2PO4 + 10.9 g Na2HPO4 + 18.5 g NaCl)

1200 g Sucrose

1200 mL Ethylene Glycol

Bring to 4000 mL final volume with PBS stock solution

Cryoprotectant Solution (6000 mL)

3000 mL of pre-made PBS stock solution (pH 7.2)

(or 3000 mL dH2O + 4.2 g NaH2PO4 + 16.4 g Na2HPO4 + 27.8 g NaCl)

1800 g Sucrose

1800 mL Ethylene Glycol

Bring to 6000 mL final volume with PBS stock solution

Phosphate-Buffered Saline (PBS) 0.05 M buffer, 0.158 M saline

1000 mL dH2O

9.72 g Sodium phosphate monobasic (NaH2PO4) [0.0115 M]

38.3 g Sodium phosphate dibasic (Na2HPO4) [0.0385 M]

64.8 g NaCl

Add above concentrate to 6000 mL dH2O to make 7000 mL stock solution

Adjust pH to 7.2, if needed

Tris-Buffered Saline (TBS) 0.05 M buffer, 0.15 M saline

1000 mL dH2O

6.8 g Trizma base

46.3 g Trizma hydrochloride

61.4 g NaCl

Add above concentrate to 6000 mL dH2O to make 7000 mL stock solution

Adjust pH to 7.4, if needed

Dilution Media (DM) 0.05 M buffer, 0.15 M saline, 0.05% Triton X

1000 mL dH2O

6.8 g Trizma base

46.3 g Trizma hydrochloride

61.4 g NaCl

3.5 mL Triton X-100

Add above concentrate to 6000 mL dH2O to make 7000 mL stock solution

Adjust pH to 7.4, if needed

4% Paraformaldehyde Solution (PFA) 0.1 M phosphate-buffered (4000 mL)

Solution A (make in 4000 mL beaker in fume hood)

160 g paraformaldehyde (weigh in fume hood)

Heat 2000 mL dH2O in microwave for ~7 minutes until ~65°C

Add heated dH2O to paraformaldehyde beaker until filled to 2000 mL line

Stir on heated stir plate, maintaining 60-65°C (do not exceed 70°C!!)

Add 10 M NaOH until solution clears (10-20 mL)

Allow solution to cool to <30°C, can place in ice water bath to speed cooling

Solution B (make in 4000 mL filter flask)

2000 mL dH2O in 4000 mL filter flask (Büchner flask)

13.6 g Sodium phosphate monobasic monohydrate (NaH2PO4 · H2O)

80.9 g Sodium phosphate dibasic heptahydrate (Na2HPO4 · 7H2O)

Vacuum filter Solution A into Solution B in fume hood using Büchner funnel and 15 cm filter

Adjust pH to 7.4, if needed

Store at 4°C for 1-2 weeks

4% Paraformaldehyde Solution (PFA) 0.1 M phosphate-buffered (2000 mL)

Solution A (make in 2000 mL beaker in a fume hood)

80 g paraformaldehyde (weigh in fume hood)

Heat 1000 mL dH2O in microwave for ~7 minutes until ~65°C

Add heated dH2O to paraformaldehyde beaker until filled to 1000 mL line

Stir on heated stir plate, maintaining 60-65°C (do not exceed 70°C!!)

Add 10 M NaOH until solution clears (5-10 mL)

Allow solution to cool to <30°C, can place in ice water bath to speed cooling

Solution B (make in 2000 mL or 4000 mL filter flask)

1000 mL dH2O

6.8 g Sodium phosphate monobasic monohydrate (NaH2PO4 · H2O)

40.4 g Sodium phosphate dibasic heptahydrate (Na2HPO4 · 7H2O)

Vacuum filter Solution A into Solution B in fume hood using Büchner funnel and 15 cm filter

Adjust pH to 7.4, if needed

Store at 4°C for 1-2 weeks

4% Zamboni's Fixative 0.1 M phosphate-buffered (4000 mL)

Solution A (make in 4000 mL beaker in a fume hood)

160 g paraformaldehyde (weigh in fume hood)

Heat 2000 mL dH2O in microwave for ~7 minutes until ~65°C

Add heated dH2O to paraformaldehyde beaker until filled to 2000 mL line

Stir on heated stir plate, maintaining 60-65°C (do not exceed 70°C!!)

Add 10 M NaOH until solution clears (10-20 mL)

Allow solution to cool to <30°C, can place in ice water bath to speed cooling

Solution B (make in 4000 mL filter flask)

1700 mL dH2O

6.6 g Sodium phosphate monobasic monohydrate (NaH2PO4 · H2O)

94.4 g Sodium phosphate dibasic heptahydrate (Na2HPO4 · 7H2O)

300 mL Picric Acid Solution

Vacuum filter Solution A into Solution B in fume hood using Büchner funnel and 15 cm filter

Adjust pH to 7.4, if needed

Store at room temperature for 1 year

4% Zamboni's Fixative 0.1 M phosphate-buffered (2000 mL)

Solution A (make in 2000 mL beaker in a fume hood)

80 g paraformaldehyde (weigh in fume hood)

Heat 1000 mL dH2O in microwave for ~7 minutes until ~65°C

Add heated dH2O to paraformaldehyde beaker until filled to 1000 mL line

Stir on heated stir plate, maintaining 60-65°C (do not exceed 70°C!!)

Add 10 M NaOH until solution clears (5-10 mL)

Allow solution to cool to <30°C, can place in ice water bath to speed cooling

Solution B (make in 2000 mL or 4000 mL filter flask)

850 mL dH2O

3.3 g Sodium phosphate monobasic monohydrate (NaH2PO4 · H2O)

47.2 g Sodium phosphate dibasic heptahydrate (Na2HPO4 · 7H2O)

150 mL Picric Acid Solution

Vacuum filter Solution A into Solution B in fume hood using Büchner funnel and 15 cm filter

Adjust pH to 7.4, if needed

Store at room temperature for 1 year