

COMMON SOLUTIONS

10× phosphate buffered saline (PBS; stock solution)

1,370 mM NaCl

30 mM KCl

160 mM Na₂HPO₄

20 mM KH₂PO₄

Adjust the pH to 7.4 with 10N sodium hydroxide, add deionized water to the final volume. Sterilize in an autoclave.

Store at room temperature (RT); after opening, store the bottle in a refrigerator.

To prepare the working solution of PBS (**1× PBS**), dilute 10 times the stock solution of PBS in deionized water. Store at 4°C.

2% (w/v) formaldehyde in 1× PBS buffer, pH 7.4 (working solution)

Mix the paraformaldehyde with deionized water (one half of the final fixative volume) in an Erlenmeyer flask. Heat to 60°C on a stir plate. When moisture forms on the sides of the flask, add sodium hydroxide (~ 8 drops of 1 N solution per 0.5 l), and stir until the solution clears. Cool the solution under the faucet. Adjust the pH if necessary and fill with deionized water to the final volume. Store at -20°C.

Formaldehyde is toxic. Use gloves, prepare and use fixatives under a fume hood.

0.2% (v/v) Triton X-100 in 1× PBS buffer, pH 7.4 (working solution)

Heat 1× PBS to ~40°C. Add 100% Triton X-100. Mix for 5 minutes on a vortex and cool to room temperature. Do not freeze.

1 M Tris-HCl, pH 8: (stock solution)

Dissolve Tris base in deionized water. Add concentrated hydrochloric acid to adjust the pH to 8 and fill with deionized water to the final volume. Store at 4°C.

1 M Tris-HCl, pH 7.5 (stock solution)

Dilute Tris base in deionized water. Add concentrated hydrochloric acid to adjust the pH to 7.5 and fill with deionized water to the final volume. Store at 4°C.

100 mM Tris-HCl, pH 7.5 (working solution)

Dilute 10 times the stock solution of Tris-HCl, pH 7.5 in deionized water. Store at 4°C.

Mounting medium for glass slides (working solution)

90% (v/v) glycerol

50 mM Tris-HCl, pH 8

Add 1,4-diazabicyclo[2.2.2]octane (DABCO) to the final concentration of 2.5% (w/v) to the prepared solution. Store at -20°C.

1 M Hepes, pH 7.4 (stock solution)

Dissolve Hepes in deionized water (4/5 of the final volume). Adjust the pH with 10 N NaOH. Fill with deionized water to the final volume. Store at 4°C.

1 M NaCl (stock solution)

Dilute sodium chloride in deionized water. Store at 4°C.

100 mM Tris-HCl, pH 7.5 with 100 mM NaCl (working solution)

Dilute 10 times the stock solution of Tris-HCl, pH 7.5 and 1 M NaCl in deionized water. Store at 4°C.

1 M PIPES

Mix the PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)) with distilled water (3/4 of the final volume), adjust the pH by adding concentrated 5 N sodium hydroxide to achieve a pH of 6.95–7.3, and fill with distilled water to the final volume.

8 mM CuSO₄ and 200 mM NaCl (Solution A)

Dissolve CuSO₄ in deionized water, add an appropriate volume of 1 M NaCl (stock solution) and fill with deionized water to the final volume. Store at 4°C.

20 mM sodium ascorbate and 40 mM glycine (Solution B)

Dissolve sodium ascorbate and glycine in deionized water and fill with deionized water to the final volume. Store at -20°C.

As an aqueous solution of sodium ascorbate is the subject of quick air oxidation, prepare the aliquots in Eppendorf tubes and seal them with parafilm.

10 mM azide dye solution (e.g. FAM azide)

Dissolve azide dye in DMSO. Store at -20°C.

8 % formaldehyde in 100 mM PIPES, pH 6.95 (working solution)

Mix the paraformaldehyde with distilled water (3/4 of the final volume). Heat to 60°C on a magnetic stir plate. When moisture forms on the sides of the beaker, add sodium hydroxide (drops of 1 N solution), and stir until the solution clears. Cool the solution under the faucet. Filter, and then add the required volume of PIPES buffer (1 M, pH 6.95). Adjust the pH if necessary and fill with distilled water to the final volume.

Formaldehyde is toxic. Use gloves, and prepare and use fixatives under a fume hood.