

**Zirconium standard solution (1 g/L Zr).** 5003500.

Dissolve *zirconyl nitrate R* equivalent to 0.293 g of  $ZrO(NO_3)_2 \cdot 2H_2O$  in a mixture of 2 volumes of *hydrochloric acid R* and 8 volumes of *water R* and dilute to 100.0 mL with the same mixture of solvents.

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**4.1.3. BUFFER SOLUTIONS****Buffered acetone solution.** 4000100.

Dissolve 8.15 g of *sodium acetate R* and 42 g of *sodium chloride R* in *water R*, add 68 mL of 0.1 M *hydrochloric acid* and 150 mL of *acetone R* and dilute to 500 mL with *water R*.

**Buffer solution pH 2.0.** 4000200.

Dissolve 6.57 g of *potassium chloride R* in *water R* and add 119.0 mL of 0.1 M *hydrochloric acid*. Dilute to 1000.0 mL with *water R*.

**Phosphate buffer solution pH 2.0.** 4007900.

Dissolve 8.95 g of *disodium hydrogen phosphate R* and 3.40 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent. If necessary adjust the pH with *phosphoric acid R*.

**Sulfate buffer solution pH 2.0.** 4008900.

Dissolve 132.1 g of *ammonium sulfate R* in *water R* and dilute to 500.0 mL with the same solvent (Solution A). Carefully and with constant cooling stir 14 mL of *sulfuric acid R* into about 400 mL of *water R*; allow to cool and dilute to 500.0 mL with *water R* (Solution B). Mix equal volumes of solutions A and B. Adjust the pH if necessary.

**Buffer solution pH 2.2.** 4010500.

Mix 6.7 mL of *phosphoric acid R* with 55.0 mL of a 40 g/L solution of *sodium hydroxide R* and dilute to 1000.0 mL with *water R*.

**Buffer solution pH 2.5.** 4000300.

Dissolve 100 g of *potassium dihydrogen phosphate R* in 800 mL of *water R*; adjust to pH 2.5 with *hydrochloric acid R* and dilute to 1000.0 mL with *water R*.

**Buffer solution pH 2.5 R1.** 4000400.

To 4.9 g of *dilute phosphoric acid R* add 250 mL of *water R*. Adjust the pH with *dilute sodium hydroxide solution R* and dilute to 500.0 mL with *water R*.

**Phosphate buffer solution pH 2.8.** 4010600.

Dissolve 7.8 g of *sodium dihydrogen phosphate R* in 900 mL of *water R*, adjust to pH 2.8 with *phosphoric acid R* and dilute to 1000 mL with the same solvent.

**Buffer solution pH 3.0.** 4008000.

Dissolve 21.0 g of *citric acid R* in 200 mL of 1 M *sodium hydroxide* and dilute to 1000 mL with *water R*. Dilute 40.3 mL of this solution to 100.0 mL with 0.1 M *hydrochloric acid*.

**0.25 M Citrate buffer solution pH 3.0.** 4012600.

Dissolve 4.8 g of *citric acid R* in 80 mL of *water R*. Adjust the pH with 1 M *sodium hydroxide* and dilute to 100.0 mL with *water R*.

**0.1 M Phosphate buffer solution pH 3.0.** 4011500.

Dissolve 12.0 g of *anhydrous sodium dihydrogen phosphate R* in *water R*, adjust the pH with *dilute phosphoric acid R1* and dilute to 1000 mL with *water R*.

**Phosphate buffer solution pH 3.0.** 4000500.

Mix 0.7 mL of *phosphoric acid R* with 100 mL of *water R*. Dilute to 900 mL with the same solvent. Adjust to pH 3.0 with *strong sodium hydroxide solution R* and dilute to 1000 mL with *water R*.

**Phosphate buffer solution pH 3.0 R1.** 4010000.

Dissolve 3.40 g of *potassium dihydrogen phosphate R* in 900 mL of *water R*. Adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000.0 mL with *water R*.

**Phosphate buffer solution pH 3.2.** 4008100.

To 900 mL of a 4 g/L solution of *sodium dihydrogen phosphate R*, add 100 mL of a 2.5 g/L solution of *phosphoric acid R*. Adjust the pH if necessary.

**Phosphate buffer solution pH 3.2 R1.** 4008500.

Adjust a 35.8 g/L solution of *disodium hydrogen phosphate R* to pH 3.2 with *dilute phosphoric acid R*. Dilute 100.0 mL of the solution to 2000.0 mL with *water R*.

**Buffer solution pH 3.5.** 4000600.

Dissolve 25.0 g of *ammonium acetate R* in 25 mL of *water R* and add 38.0 mL of *hydrochloric acid R1*. Adjust the pH if necessary with *dilute hydrochloric acid R* or *dilute ammonia R1*. Dilute to 100.0 mL with *water R*.

**Phosphate buffer solution pH 3.5.** 4000700.

Dissolve 68.0 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent. Adjust the pH with *phosphoric acid R*.

**Buffer solution pH 3.6.** 4000800.

To 250.0 mL of 0.2 M *potassium hydrogen phthalate R* add 11.94 mL of 0.2 M *hydrochloric acid*. Dilute to 1000.0 mL with *water R*.

**Buffer solution pH 3.7.** 4000900.

To 15.0 mL of *acetic acid R* add 60 mL of *ethanol (96 per cent) R* and 20 mL of *water R*. Adjust to pH 3.7 by the addition of *ammonia R*. Dilute to 100.0 mL with *water R*.

**Buffered copper sulfate solution pH 4.0.** 4001000.

Dissolve 0.25 g of *copper sulfate R* and 4.5 g of *ammonium acetate R* in *dilute acetic acid R* and dilute to 100.0 mL with the same solvent.

**Sodium acetate buffer solution pH 4.0 (0.1 M).** 4013800.

Dissolve 822 mg of *sodium acetate R* in 100 mL of *water R* (solution A). Dilute 1.44 mL of *glacial acetic acid R* in 250 mL of *water R* (solution B). Titrate 100 mL of solution B using about 20 mL of solution A.

**Acetate buffer solution pH 4.4.** 4001100.

Dissolve 136 g of *sodium acetate R* and 77 g of *ammonium acetate R* in *water R* and dilute to 1000.0 mL with the same solvent; add 250.0 mL of *glacial acetic acid R* and mix.

**Phthalate buffer solution pH 4.4.** 4001200.

Dissolve 2.042 g of *potassium hydrogen phthalate R* in 50 mL of *water R*, add 7.5 mL of 0.2 M *sodium hydroxide* and dilute to 200.0 mL with *water R*.

**Acetate buffer solution pH 4.5.** 4012500.

Dissolve 77.1 g of *ammonium acetate R* in *water R*. Add 70 mL of *glacial acetic acid R* and dilute to 1000.0 mL with *water R*.

**0.05 M Phosphate buffer solution pH 4.5.** 4009000.

Dissolve 6.80 g of *potassium dihydrogen phosphate R* in 1000.0 mL of *water R*. The pH (2.2.3) of the solution is 4.5.

**Sodium acetate buffer solution pH 4.5.** 4010100.

Dissolve 63 g of *anhydrous sodium acetate R* in *water R*, add 90 mL *acetic acid R* and adjust to pH 4.5, and dilute to 1000 mL with *water R*.

**Acetate buffer solution pH 4.6.** 4001400.

Dissolve 5.4 g of *sodium acetate R* in 50 mL of *water R*, add 2.4 g of *glacial acetic acid R* and dilute to 100.0 mL with *water R*. Adjust the pH if necessary.

**Succinate buffer solution pH 4.6.** 4001500.

Dissolve 11.8 g of *succinic acid R* in a mixture of 600 mL of *water R* and 82 mL of 1 M *sodium hydroxide* and dilute to 1000.0 mL with *water R*.

**Acetate buffer solution pH 4.7.** 4001600.

Dissolve 136.1 g of *sodium acetate R* in 500 mL of *water R*. Mix 250 mL of this solution with 250 mL of *dilute acetic acid R*. Shake twice with a freshly prepared, filtered, 0.1 g/L solution of *dithizone R* in *chloroform R*. Shake with *carbon tetrachloride R* until the extract is colourless. Filter the aqueous layer to remove traces of carbon tetrachloride.

**Acetate buffer solution pH 4.7 R1.** 4013600.

Dissolve 136.1 g of *sodium acetate R* in 500 mL of *water R*. Mix 250 mL of this solution with 250 mL of *dilute acetic acid R*.

**Acetate buffer solution pH 5.0.** 4009100.

To 120 mL of a 6 g/L solution of *glacial acetic acid R* add 100 mL of 0.1 M *potassium hydroxide* and about 250 mL of *water R*. Mix. Adjust the pH to 5.0 with a 6 g/L solution of *acetic acid R* or with 0.1 M *potassium hydroxide* and dilute to 1000.0 mL with *water R*.

**Citrate buffer solution pH 5.0.** 4010700.

Prepare a solution containing 20.1 g/L of *citric acid R* and 8.0 g/L of *sodium hydroxide R*. Adjust the pH with *dilute hydrochloric acid R*.

**Phosphate buffer solution pH 5.0.** 4011300.

Dissolve 2.72 g of *potassium dihydrogen phosphate R* in 800 mL of *water R*. Adjust the pH with 1 M *potassium hydroxide* and dilute to 1000 mL with *water R*.

**Buffer solution pH 5.2.** 4001700.

Dissolve 1.02 g of *potassium hydrogen phthalate R* in 30.0 mL of 0.1 M *sodium hydroxide*. Dilute to 100.0 mL with *water R*.

**0.067 M Phosphate buffer solution pH 5.4.** 4012000.

Mix appropriate volumes of a 23.99 g/L solution of *disodium hydrogen phosphate R* with a 9.12 g/L solution of *sodium dihydrogen phosphate monohydrate R* to obtain pH 5.4 (2.2.3).

**Acetate-edetate buffer solution pH 5.5.** 4001900.

Dissolve 250 g of *ammonium acetate R* and 15 g *sodium edetate R* in 400 mL of *water R* and add 125 mL of *glacial acetic acid R*.

**Buffer solution pH 5.5.** 4001800.

Dissolve 54.4 g of *sodium acetate R* in 50 mL of *water R*, heating to 35 °C if necessary. After cooling, slowly add 10 mL of *anhydrous acetic acid R*. Shake and dilute to 100.0 mL with *water R*.

**Phosphate buffer solution pH 5.5.** 4002000.

Dissolve 13.61 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent (solution A). Dissolve 35.81 g of *disodium hydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent (solution B). Mix 96.4 mL of solution A and 3.6 mL of solution B.

**Phosphate-citrate buffer solution pH 5.5.** 4008700.

Mix 56.85 mL of a 28.4 g/L solution of *anhydrous disodium hydrogen phosphate R* and 43.15 mL of a 21 g/L solution of *citric acid R*.

**Phosphate buffer solution pH 5.6.** 4011200.

Dissolve 0.908 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 100.0 mL with the same solvent (solution A). Dissolve 1.161 g of *dipotassium hydrogen phosphate R* in *water R* and dilute to 100.0 mL with the same solvent (solution B). Mix 94.4 mL of solution A and 5.6 mL of solution B. If necessary, adjust to pH 5.6 using solution A or solution B.

**Phosphate buffer solution pH 5.8.** 4002100.

Dissolve 1.19 g of *disodium hydrogen phosphate dihydrate R* and 8.25 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent.

**Acetate buffer solution pH 6.0.** 4002200.

Dissolve 100 g of *ammonium acetate R* in 300 mL of *water R*, add 4.1 mL of *glacial acetic acid R*, adjust the pH if necessary using *ammonia R* or *acetic acid R* and dilute to 500.0 mL with *water R*.

**Diethylammonium phosphate buffer solution pH 6.0.** 4002300.

Dilute 68 mL of *phosphoric acid R* to 500 mL with *water R*. To 25 mL of this solution add 450 mL of *water R* and 6 mL of *diethylamine R*, adjust to pH 6 ± 0.05, if necessary, using *diethylamine R* or *phosphoric acid R* and dilute to 500.0 mL with *water R*.

**Phosphate buffer solution pH 6.0.** 4002400.

Mix 63.2 mL of a 71.5 g/L solution of *disodium hydrogen phosphate R* and 36.8 mL of a 21 g/L solution of *citric acid R*.

**Phosphate buffer solution pH 6.0 R1.** 4002500.

Dissolve 6.8 g of *sodium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with *water R*. Adjust the pH with *strong sodium hydroxide solution R*.

**Phosphate buffer solution pH 6.0 R2.** 4002600.

To 250.0 mL of 0.2 M *potassium dihydrogen phosphate R* add 28.5 mL of 0.2 M *sodium hydroxide* and dilute to 1000.0 mL with *water R*.

**Phosphate buffer solution pH 6.4.** 4002800.

Dissolve 2.5 g of *disodium hydrogen phosphate R*, 2.5 g of *sodium dihydrogen phosphate R* and 8.2 g of *sodium chloride R* in 950 mL of *water R*. Adjust the pH of the solution to 6.4 with 1 M *sodium hydroxide* or 1 M *hydrochloric acid*, if necessary. Dilute to 1000.0 mL with *water R*.

**0.5 M Phthalate buffer solution pH 6.4.** 4009200.

Dissolve 100 g of *potassium hydrogen phthalate R* in *water R* and dilute to 1000.0 mL with the same solvent. Adjust the pH if necessary, using *strong sodium hydroxide solution R*.

**Buffer solution pH 6.5.** 4002900.

Dissolve 60.5 g of *disodium hydrogen phosphate R* and 46 g of *potassium dihydrogen phosphate R* in *water R*. Add 100 mL of 0.02 M *sodium edetate* and 20 mg of *mercuric chloride R* and dilute to 1000.0 mL with *water R*.

**Imidazole buffer solution pH 6.5.** 4003000.

Dissolve 6.81 g of *imidazole R*, 1.23 g of *magnesium sulfate R* and 0.73 g of *calcium sulfate R* in 752 mL of 0.1 M *hydrochloric acid*. Adjust the pH if necessary and dilute to 1000.0 mL with *water R*.

**0.1 M phosphate buffer solution pH 6.5.** 4010800.

Dissolve 13.80 g of *sodium dihydrogen phosphate monohydrate R* in 900 mL of *distilled water R*. Adjust the pH using a 400 g/L solution of *sodium hydroxide R*. Dilute to 1000 mL with *distilled water R*.

**Phosphate buffer solution pH 6.5.** 4012800.

Dissolve 2.75 g of *sodium dihydrogen phosphate R* and 4.5 g of *sodium chloride R* in 500 mL of *water R*. Adjust the pH with *phosphate buffer solution pH 8.5 R*.

**Buffer solution pH 6.6.** 4003100.

To 250.0 mL of 0.2 M *potassium dihydrogen phosphate R* add 89.0 mL of 0.2 M *sodium hydroxide*. Dilute to 1000.0 mL with *water R*.

**Phosphate buffered saline pH 6.8.** 4003200.

Dissolve 1.0 g of *potassium dihydrogen phosphate R*, 2.0 g of *dipotassium hydrogen phosphate R* and 8.5 g of *sodium chloride R* in 900 mL of *water R*, adjust the pH if necessary and dilute to 1000.0 mL with the same solvent.

**Phosphate buffer solution pH 6.8.** 4003300.

Mix 77.3 mL of a 71.5 g/L solution of *disodium hydrogen phosphate R* with 22.7 mL of a 21 g/L solution of *citric acid R*.

**Phosphate buffer solution pH 6.8 R1.** 4003400.

To 51.0 mL of a 27.2 g/L solution of *potassium dihydrogen phosphate R* add 49.0 mL of a 71.6 g/L solution of *disodium hydrogen phosphate R*. Adjust the pH if necessary.

*Storage:* at 2 °C to 8 °C.

**1 M tris-hydrochloride buffer solution pH 6.8.** 4009300.

Dissolve 60.6 g of *tris(hydroxymethyl)aminomethane R* in 400 mL of *water R*. Adjust the pH with *hydrochloric acid R* and dilute to 500.0 mL with *water R*.

**Buffer solution pH 7.0.** 4003500.

To 1000 mL of a solution containing 18 g/L of *disodium hydrogen phosphate R* and 23 g/L of *sodium chloride R* add sufficient (about 280 mL) of a solution containing 7.8 g/L of *sodium dihydrogen phosphate R* and 23 g/L of *sodium chloride R* to adjust the pH. Dissolve in the solution sufficient *sodium azide R* to give a 0.2 g/L solution.

**Maleate buffer solution pH 7.0.** 4003600.

Dissolve 10.0 g of *sodium chloride R*, 6.06 g of *tris(hydroxymethyl)aminomethane R* and 4.90 g of *maleic anhydride R* in 900 mL of *water R*. Adjust the pH using a 170 g/L solution of *sodium hydroxide R*. Dilute to 1000.0 mL with *water R*.

*Storage:* at 2 °C to 8 °C; use within 3 days.

**0.025 M Phosphate buffer solution pH 7.0.** 4009400.

Mix 1 volume of 0.063 M phosphate buffer solution pH 7.0 R with 1.5 volumes of *water R*.

**0.03 M Phosphate buffer solution pH 7.0.** 4010300.

Dissolve 5.2 g of *dipotassium hydrogen phosphate R* in 900 mL of *water for chromatography R*. Adjust the solution to pH 7.0 ± 0.1 using *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

**0.05 M Phosphate buffer solution pH 7.0.** 4012400.

Mix 34 mL of *water R* and 100 mL of 0.067 M phosphate buffer solution pH 7.0 R.

**0.063 M Phosphate buffer solution pH 7.0.** 4009500.

Dissolve 5.18 g of *anhydrous disodium hydrogen phosphate R* and 3.65 g of *sodium dihydrogen phosphate monohydrate R* in 950 mL of *water R* and adjust the pH with *phosphoric acid R*; dilute to 1000.0 mL with *water R*.

**0.067 M Phosphate buffer solution pH 7.0.** 4003800.

Dissolve 0.908 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 100.0 mL with the same solvent (solution A). Dissolve 2.38 g of *disodium hydrogen phosphate R* in *water R* and dilute to 100.0 mL with the same solvent (solution B). Mix 38.9 mL of solution A and 61.1 mL of solution B. Adjust the pH if necessary.

**0.1 M Phosphate buffer solution pH 7.0.** 4008200.

Dissolve 1.361 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 100.0 mL with the same solvent. Adjust the pH using a 35 g/L solution of *disodium hydrogen phosphate R*.

**Phosphate buffer solution pH 7.0.** 4003700.

Mix 82.4 mL of a 71.5 g/L solution of *disodium hydrogen phosphate R* with 17.6 mL of a 21 g/L solution of *citric acid R*.

**Phosphate buffer solution pH 7.0 R1.** 4003900.

Mix 250.0 mL of 0.2 M *potassium dihydrogen phosphate R* and 148.2 mL of a 8 g/L solution of *sodium hydroxide R*, adjust the pH if necessary. Dilute to 1000.0 mL with *water R*.

**Phosphate buffer solution pH 7.0 R2.** 4004000.

Mix 50.0 mL of a 136 g/L solution of *potassium dihydrogen phosphate R* with 29.5 mL of 1 M *sodium hydroxide* and dilute to 100.0 mL with *water R*. Adjust the pH to 7.0 ± 0.1.

**Phosphate buffer solution pH 7.0 R3.** 4008600.

Dissolve 5 g of *potassium dihydrogen phosphate R* and 11 g of *dipotassium hydrogen phosphate R* in 900 mL of *water R*. Adjust to pH 7.0 with *dilute phosphoric acid R* or *dilute sodium hydroxide solution R*. Dilute to 1000 mL with *water R* and mix.

**Phosphate buffer solution pH 7.0 R4.** 4010200.

Dissolve 28.4 g of *anhydrous disodium hydrogen phosphate R* and 18.2 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 500 mL with the same solvent.

**Phosphate buffer solution pH 7.0 R5.** 4011400.

Dissolve 28.4 g of *anhydrous disodium hydrogen phosphate R* in 800 mL of *water R*. Adjust the pH using a 30 per cent *m/m* solution of *phosphoric acid R* and dilute to 1000 mL with *water R*.

**Tetrabutylammonium buffer solution pH 7.0.** 4010900.

Dissolve 6.16 g of *ammonium acetate R* in a mixture of 15 mL of *tetrabutylammonium hydroxide solution (400 g/L) R* and 185 mL of *water R*. Adjust the pH with *nitric acid R*.

**Buffered salt solution pH 7.2.** 4004300.

Dissolve in *water R* 8.0 g of *sodium chloride R*, 0.2 g of *potassium chloride R*, 0.1 g of *anhydrous calcium chloride R*, 0.1 g of *magnesium chloride R*, 3.18 g of *disodium hydrogen phosphate R* and 0.2 g of *potassium dihydrogen phosphate R* and dilute to 1000.0 mL with *water R*.

**Buffer solution pH 7.2.** 4004100.

To 250.0 mL of 0.2 M *potassium dihydrogen phosphate R* add 175.0 mL of 0.2 M *sodium hydroxide*. Dilute to 1000.0 mL with *water R*. Adjust the pH if necessary.

**Phosphate-albumin buffered saline pH 7.2.** 4004400.

Dissolve 10.75 g of *disodium hydrogen phosphate R*, 7.6 g of *sodium chloride R* and 10 g of *bovine albumin R* in *water R* and dilute to 1000.0 mL with the same solvent. Immediately before use adjust the pH using *dilute sodium hydroxide solution R* or *dilute phosphoric acid R*.

**Phosphate-albumin buffered saline pH 7.2 R1.** 4009600.

Dissolve 10.75 g of *disodium hydrogen phosphate R*, 7.6 g of *sodium chloride R* and 1 g of *bovine albumin R* in *water R* and dilute to 1000.0 mL with the same solvent. Immediately before use adjust the pH using *dilute sodium hydroxide solution R* or *dilute phosphoric acid R*.

**Phosphate buffer solution pH 7.2.** 4004200.

Mix 87.0 mL of a 71.5 g/L solution of *disodium hydrogen phosphate R* with 13.0 mL of a 21 g/L solution of *citric acid R*.

**Imidazole buffer solution pH 7.3.** 4004500.

Dissolve 3.4 g of *imidazole R* and 5.8 g of *sodium chloride R* in *water R*, add 18.6 mL of 1 M *hydrochloric acid* and dilute to 1000.0 mL with *water R*. Adjust the pH if necessary.

**Barbital buffer solution pH 7.4.** 4004700.

Mix 50 mL of a solution in *water R* containing 19.44 g/L of *sodium acetate R* and 29.46 g/L of *barbital sodium R* with 50.5 mL of 0.1 M *hydrochloric acid*, add 20 mL of an 85 g/L of *sodium chloride R* and dilute to 250 mL with *water R*.

**Buffer solution pH 7.4.** 4004600.

Dissolve 0.6 g of *potassium dihydrogen phosphate R*, 6.4 g of *disodium hydrogen phosphate R* and 5.85 g of *sodium chloride R* in *water R*, and dilute to 1000.0 mL with the same solvent. Adjust the pH if necessary.

**Phosphate buffered saline pH 7.4.** 4005000.

Dissolve 2.38 g of *disodium hydrogen phosphate R*, 0.19 g of *potassium dihydrogen phosphate R* and 8.0 g of *sodium chloride R* in *water R*. Dilute to 1000.0 mL with the same solvent. Adjust the pH if necessary.

**Phosphate buffer solution pH 7.4.** 4004800.

Add 250.0 mL of 0.2 M *potassium dihydrogen phosphate R* to 393.4 mL of 0.1 M *sodium hydroxide*.

**Tris(hydroxymethyl)aminomethane buffer solution pH 7.4.** 4012100.

Dissolve 30.3 g of *tris(hydroxymethyl)aminomethane R* in approximately 200 mL of *water R*. Add 183 mL of 1 M *hydrochloric acid*. Dilute to 500.0 mL with *water R*. Note: the pH is 7.7-7.8 at room temperature and 7.4 at 37 °C. This solution is stable for several months at 4 °C.

**Tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4.** 4004900.

Dissolve 6.08 g of *tris(hydroxymethyl)aminomethane R*, 8.77 g of *sodium chloride R* in 500 mL of *distilled water R*. Add 10.0 g of *bovine albumin R*. Adjust the pH using *hydrochloric acid R*. Dilute to 1000.0 mL with *distilled water R*.

**Tris(hydroxymethyl)aminomethane sodium chloride buffer solution pH 7.4 R1.** 4012200.

Dissolve 0.1 g of *bovine albumin R* in a mixture containing 2 mL of *tris(hydroxymethyl)aminomethane buffer solution pH 7.4 R* and 50 mL of a 5.84 mg/mL solution of *sodium chloride R*. Dilute to 100.0 mL with *water R*.

**Tris-sodium acetate buffer solution pH 7.4.** 4012900.

Dissolve 6.3 g of *tris(hydroxymethyl)aminomethane R* and 4.9 g of *anhydrous sodium acetate R* in 900 mL of *water R*. Adjust to pH 7.4 with *sulfuric acid R* and dilute to 1000 mL with *water R*.

**Tris-sodium acetate-sodium chloride buffer solution pH 7.4.** 4013000.

Dissolve 30.0 g of *tris(hydroxymethyl)aminomethane R*, 14.5 g of *anhydrous sodium acetate R* and 14.6 g of *sodium chloride R* in 900 mL of *water R*. Add 0.50 g of *bovine albumin R*. Adjust to pH 7.4 with *sulfuric acid R* and dilute to 1000 mL with *water R*.

**Borate buffer solution pH 7.5.** 4005200.

Dissolve 2.5 g of *sodium chloride R*, 2.85 g of *disodium tetraborate R* and 10.5 g of *boric acid R* in *water R* and dilute to 1000.0 mL with the same solvent. Adjust the pH if necessary. Storage: at 2 °C to 8 °C.

**Buffer (HEPES) solution pH 7.5.** 4009700.

Dissolve 2.38 g of 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid *R* in about 90 mL of *water R*. Adjust the pH to 7.5 with *sodium hydroxide solution R*. Dilute to 100 mL with *water R*.

**0.2 M Phosphate buffer solution pH 7.5.** 4005400.

Dissolve 27.22 g of *potassium dihydrogen phosphate R* in 930 mL of *water R*, adjust to pH 7.5 with a 300 g/L solution of *potassium hydroxide R* and dilute to 1000.0 mL with *water R*.

**0.33 M Phosphate buffer solution pH 7.5.** 4005300.

Dissolve 119.31 g of *disodium hydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent (solution A). Dissolve 45.36 g of *potassium dihydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent (solution B). Mix 85 mL of solution A and 15 mL of solution B. Adjust the pH if necessary.

**0.05 M Tris-hydrochloride buffer solution pH 7.5.** 4005600.

Dissolve 6.057 g of *tris(hydroxymethyl)aminomethane R* in *water R* and adjust the pH with *hydrochloric acid R*. Dilute to 1000.0 mL with *water R*.

**Tris(hydroxymethyl)aminomethane buffer solution pH 7.5.** 4005500.

Dissolve 7.27 g of *tris(hydroxymethyl)aminomethane R* and 5.27 g of *sodium chloride R* in *water R*, and adjust the pH if necessary. Dilute to 1000.0 mL with *water R*.

**Sodium citrate buffer solution pH 7.8 (0.034 M sodium citrate, 0.101 M sodium chloride).** 4009800.

Dissolve 10.0 g of *sodium citrate R* and 5.90 g of *sodium chloride R* in 900 mL of *water R*. Adjust the pH by addition of *hydrochloric acid R* and dilute to 1000 mL with *water R*.

**0.0015 M Borate buffer solution pH 8.0.** 4006000.

Dissolve 0.572 g of *disodium tetraborate R* and 2.94 g of *calcium chloride R* in 800 mL of *water R*. Adjust the pH with 1 M *hydrochloric acid*. Dilute to 1000.0 mL with *water R*.

**Buffer solution pH 8.0.** 4005900.

To 50.0 mL of 0.2 M *potassium dihydrogen phosphate R* add 46.8 mL of 0.2 M *sodium hydroxide*. Dilute to 200.0 mL with *water R*.

**Buffer solution pH 8.0 R1.** 4010400.

Dissolve 20 g of *dipotassium hydrogen phosphate R* in 900 mL of *water R*. Adjust the pH with *phosphoric acid R*. Dilute to 1000 mL with *water R*.

**0.02 M Phosphate buffer solution pH 8.0.** 4006100.

To 50.0 mL of 0.2 M *potassium dihydrogen phosphate R* add 46.8 mL of 0.2 M *sodium hydroxide*. Dilute to 500.0 mL with *water R*.

**0.02 M Sodium phosphate buffer solution pH 8.0.** 4013700.

Dissolve 0.31 g of *sodium dihydrogen phosphate R* in 70 mL of *water R* and adjust to pH 8.0 with 1 M *sodium hydroxide*, then dilute to 100 mL with *water R*.

**0.1 M Phosphate buffer solution pH 8.0.** 4008400.

Dissolve 0.523 g of *potassium dihydrogen phosphate R* and 16.73 g of *dipotassium hydrogen phosphate R* in *water R* and dilute to 1000.0 mL with the same solvent.

**1 M Phosphate buffer solution pH 8.0.** 4007800.

Dissolve 136.1 g of *potassium dihydrogen phosphate R* in *water R*, adjust the pH with 1 M *sodium hydroxide*. Dilute to 1000.0 mL with *water R*.

**1 M Tris-hydrochloride buffer solution pH 8.0.** 4012700.

Dissolve 121.1 g of *tris(hydroxymethyl)aminomethane R* and 1.47 g of *calcium chloride R* in 900 mL of *water R*. Adjust the pH with *hydrochloric acid R* and dilute to 1000.0 mL with *water R*.

**Tris-hydrochloride buffer solution pH 8.0.** 4012300.

Dissolve 1.21 g of *tris(hydroxymethyl)aminomethane R* and 29.4 mg of *calcium chloride R* in *water R*. Adjust the pH with 1 M *hydrochloric acid* and dilute to 100.0 mL with *water R*.

**Tris-sodium acetate buffer solution pH 8.0.** 4013100.

Dissolve 6.3 g of *tris(hydroxymethyl)aminomethane R* and 4.9 g of *anhydrous sodium acetate R* in 900 mL of *water R*. Adjust to pH 8.0 with *sulfuric acid R* and dilute to 1000 mL with *water R*.

**Tris-sodium acetate-sodium chloride buffer solution pH 8.0.** 4013200.

Dissolve 30.0 g of *tris(hydroxymethyl)aminomethane R*, 14.5 g of *anhydrous sodium acetate R* and 14.6 g of *sodium chloride R* in 900 mL of *water R*. Add 0.50 g of *bovine albumin R*. Adjust to pH 8.0 with *sulfuric acid R* and dilute to 1000 mL with *water R*.

**Tris(hydroxymethyl)aminomethane buffer solution pH 8.1.** 4006200.

Dissolve 0.294 g of *calcium chloride R* in 40 mL of *tris(hydroxymethyl)aminomethane solution R* and adjust the pH with *1 M hydrochloric acid*. Dilute to 100.0 mL with *water R*.

**Tris-glycine buffer solution pH 8.3.** 4006300.

Dissolve 6.0 g of *tris(hydroxymethyl)aminomethane R* and 28.8 g of *glycine R* in *water R* and dilute to 1000.0 mL with the same solvent. Dilute 1 volume to 10 volumes with *water R* immediately before use.

**Tris-hydrochloride buffer solution pH 8.3.** 4011800.

Dissolve 9.0 g of *tris(hydroxymethyl)aminomethane R* in 2.9 L of *water R*. Adjust the pH with *1 M hydrochloric acid*. Adjust the volume to 3 L with *water R*.

**0.05 M Tris-hydrochloride buffer solution pH 9.0.** 4013500.

Dissolve 0.605 g of *tris(hydroxymethyl)aminomethane R* in *water R*. Adjust the pH with *1 M hydrochloric acid* and dilute to 100.0 mL with *water R*.

**Barbital buffer solution pH 8.4.** 4006400.

Dissolve 8.25 g of *barbital sodium R* in *water R* and dilute to 1000.0 mL with the same solvent.

**Tris-EDTA BSA buffer solution pH 8.4.** 4006500.

Dissolve 6.1 g of *tris(hydroxymethyl)aminomethane R*, 2.8 g of *sodium edetate R*, 10.2 g of *sodium chloride R* and 10 g of *bovine albumin R* in *water R*, adjust to pH 8.4 using *1 M hydrochloric acid* and dilute to 1000.0 mL with *water R*.

**Tris(hydroxymethyl)aminomethane-EDTA buffer solution pH 8.4.** 4006600.

Dissolve 5.12 g of *sodium chloride R*, 3.03 g of *tris(hydroxymethyl)aminomethane R* and 1.40 g of *sodium edetate R* in 250 mL of *distilled water R*. Adjust the pH to 8.4 using *hydrochloric acid R*. Dilute to 500.0 mL with *distilled water R*.

**Phosphate buffer solution pH 8.5.** 4013300.

Dissolve 3.5 g of *dipotassium hydrogen phosphate R* and 4.5 g of *sodium chloride R* in 500 mL of *water R*. Adjust the pH with a mixture of equal volumes of *dilute phosphoric acid R* and *water R*.

**Tris acetate buffer solution pH 8.5.** 4006700.

Dissolve 0.294 g of *calcium chloride R* and 12.11 g of *tris(hydroxymethyl)aminomethane R* in *water R*. Adjust the pH with *acetic acid R*. Dilute to 1000.0 mL with *water R*.

**Barbital buffer solution pH 8.6 R1.** 4006900.

Dissolve in *water R* 1.38 g of *barbital R*, 8.76 g of *barbital sodium R* and 0.38 g of *calcium lactate R* and dilute to 1000.0 mL with the same solvent.

**1.5 M tris-hydrochloride buffer solution pH 8.8.** 4009900.

Dissolve 90.8 g of *tris(hydroxymethyl)aminomethane R* in 400 mL of *water R*. Adjust the pH with *hydrochloric acid R* and dilute to 500.0 mL with *water R*.

**Buffer (phosphate) solution pH 9.0.** 4008300.

Dissolve 1.74 g of *potassium dihydrogen phosphate R* in 80 mL of *water R*, adjust the pH with *1 M potassium hydroxide* and dilute to 100.0 mL with *water R*.

**Buffer solution pH 9.0.** 4007000.

Dissolve 6.18 g of *boric acid R* in *0.1 M potassium chloride R* and dilute to 1000.0 mL with the same solvent. Mix 1000.0 mL of this solution and 420.0 mL of *0.1 M sodium hydroxide*.

**Buffer solution pH 9.0 R1.** 4007100.

Dissolve 6.20 g of *boric acid R* in 500 mL of *water R* and adjust the pH with *1 M sodium hydroxide* (about 41.5 mL). Dilute to 1000.0 mL with *water R*.

**Ammonium chloride buffer solution pH 9.5.** 4007200.

Dissolve 33.5 g of *ammonium chloride R* in 150 mL of *water R*, add 42.0 mL of *concentrated ammonia R* and dilute to 250.0 mL with *water R*.

*Storage:* in a polyethylene container.

**Ammonium chloride buffer solution pH 10.0.** 4007300.

Dissolve 5.4 g of *ammonium chloride R* in 20 mL of *water R*, add 35.0 mL of *ammonia R* and dilute to 100.0 mL with *water R*.

**Diethanolamine buffer solution pH 10.0.** 4007500.

Dissolve 96.4 g of *diethanolamine R* in *water R* and dilute to 400 mL with the same solvent. Add 0.5 mL of an 186 g/L solution of *magnesium chloride R* and adjust the pH with *1 M hydrochloric acid*. Dilute to 500.0 mL with *water R*.

**0.1 M Ammonium carbonate buffer solution pH 10.3.** 4011900.

Dissolve 7.91 g of *ammonium carbonate R* in 800 mL of *water R*. Adjust the pH with *dilute sodium hydroxide solution R*. Dilute to 1000.0 mL with *water R*.

**Ammonium chloride buffer solution pH 10.4.** 4011000.

Dissolve 70 g of *ammonium chloride R* in 200 mL of *water R*, add 330 mL of *concentrated ammonia R* and dilute to 1000.0 mL with *water R*. If necessary, adjust to pH 10.4 with *ammonia R*.

**Borate buffer solution pH 10.4.** 4011100.

Dissolve 24.64 g of *boric acid R* in 900 mL of *distilled water R*. Adjust the pH using a 400 g/L solution of *sodium hydroxide R*. Dilute to 1000 mL with *distilled water R*.

**Ammonium chloride buffer solution pH 10.7.** 4013400.

Dissolve 67.5 g of *ammonium chloride R* in *water R*, add 570 mL of *concentrated ammonia R* and dilute to 1000.0 mL with *water R*.

**Buffer solution pH 10.9.** 4007600.

Dissolve 6.75 g of *ammonium chloride R* in *ammonia R* and dilute to 100.0 mL with the same solvent.

**Total-ionic-strength-adjustment buffer.** 4007700.

Dissolve 58.5 g of *sodium chloride R*, 57.0 mL of *glacial acetic acid R*, 61.5 g of *sodium acetate R* and 5.0 g of *cyclohexylene-dinitrilotetra-acetic acid R* in *water R* and dilute to 500.0 mL with the same solvent. Adjust to pH 5.0 to 5.5 with a 335 g/L solution of *sodium hydroxide R* and dilute to 1000.0 mL with *distilled water R*.

**Total-ionic-strength-adjustment buffer R1.** 4008800.

Dissolve 210 g of *citric acid R* in 400 mL of *distilled water R*. Adjust to pH 7.0 with *concentrated ammonia R*. Dilute to 1000.0 mL with *distilled water R* (solution A). Dissolve 132 g of *ammonium phosphate R* in *distilled water R* and dilute to 1000.0 mL with the same solvent (solution B). To a suspension of 292 g of (ethylenedinitrilo)tetra-acetic acid R in about 500 mL of *distilled water R*, add about 200 mL of *concentrated ammonia R* to dissolve. Adjust the pH to 6 to

7 with *concentrated ammonia R*. Dilute to 1000.0 mL with *distilled water R* (solution C). Mix equal volumes of solution A, B, and C and adjust to pH 7.5 with *concentrated ammonia R*.

## 4.2. VOLUMETRIC ANALYSIS

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### 4.2.1. PRIMARY STANDARDS FOR VOLUMETRIC SOLUTIONS

Primary standards for volumetric solutions are indicated by the suffix RV. Primary standards of suitable quality may be obtained from commercial sources or prepared by the following methods.

**Arsenious trioxide.**  $\text{As}_2\text{O}_3$ . ( $M_r$  197.8). 2000100. [1327-53-3].  
Sublime *arsenious trioxide R* in a suitable apparatus.  
*Storage*: over *anhydrous silica gel R*.

**Benzoic acid.**  $\text{C}_7\text{H}_6\text{O}_2$ . ( $M_r$  122.1). 2000200. [65-85-0].  
Sublime *benzoic acid R* in a suitable apparatus.

**Potassium bromate.**  $\text{KBrO}_3$ . ( $M_r$  167.0). 2000300. [7758-01-2].  
Crystallise *potassium bromate R* from boiling *water R*. Collect the crystals and dry to constant mass at 180 °C.

**Potassium hydrogen phthalate.**  $\text{C}_8\text{H}_5\text{KO}_4$ . ( $M_r$  204.2). 2000400. [877-24-7].

Recrystallise *potassium hydrogen phthalate R* from boiling *water R*, collect the crystals at a temperature above 35 °C and dry to constant mass at 110 °C.

**Sodium carbonate.**  $\text{Na}_2\text{CO}_3$ . ( $M_r$  106.0). 2000500. [497-19-8].  
Filter at room temperature a saturated solution of *sodium carbonate R*. Introduce slowly into the filtrate a stream of *carbon dioxide R* with constant cooling and stirring. After about 2 h, collect the precipitate on a sintered-glass filter (2.1.2). Wash the filter with iced *water R* containing carbon dioxide. After drying at 100 °C to 105 °C, heat to constant mass at 270-300 °C, stirring from time to time.

**Sodium chloride.**  $\text{NaCl}$ . ( $M_r$  58.44). 2000600. [7647-14-5].  
To 1 volume of the *saturated solution of sodium chloride R* add 2 volumes of *hydrochloric acid R*. Collect the crystals formed and wash with *hydrochloric acid RI*. Remove the hydrochloric acid by heating on a water-bath and dry the crystals to constant mass at 300 °C.

**Sulfanilic acid.**  $\text{C}_6\text{H}_7\text{NO}_3\text{S}$ . ( $M_r$  173.2). 2000700. [121-57-3].  
Recrystallise *sulfanilic acid R* from boiling *water R*. Filter and dry to constant mass at 100-105 °C.

**Zinc.**  $\text{Zn}$ . ( $M_r$  65.4). 2000800. [7440-66-6].  
*Content*: minimum 99.9 per cent.

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### 4.2.2. VOLUMETRIC SOLUTIONS

Volumetric solutions are prepared according to the usual chemical analytical methods. The accuracy of the apparatus used is verified to ensure that it is appropriate for the intended use.

The concentration of volumetric solutions is indicated in terms of molarity. Molarity expresses, as the number of moles, the amount of substance dissolved in 1 L of solution. A solution which contains  $x$  moles of substance per litre is said to be  $x$  M.

Volumetric solutions do not differ from the prescribed strength by more than 10 per cent. The molarity of the volumetric solutions is determined by an appropriate number of titrations. The repeatability does not exceed 0.2 per cent (relative standard deviation).

Volumetric solutions are standardised by the methods described below. When a volumetric solution is to be used in an assay in which the end-point is determined by an electrochemical process (for example, amperometry or potentiometry) the solution is standardised by the same method. The composition of the medium in which a volumetric solution is standardised should be the same as that in which it is to be used.

Solutions more dilute than those described are obtained by dilution with *carbon dioxide-free water R* of the least-concentrated solution that describes a standardisation. The correction factors of these solutions are the same as those from which the dilutions were prepared.

#### 0.1 M Acetic acid. 3008900.

Dilute 6.0 g of *glacial acetic acid R* to 1000.0 mL with *water R*.  
*Standardisation*. To 25.0 mL of acetic acid add 0.5 mL of *phenolphthalein solution R* and titrate with 0.1 M *sodium hydroxide*.

#### 0.1 M Ammonium and cerium nitrate. 3000100.

Shake for 2 min a solution containing 56 mL of sulfuric acid R and 54.82 g of *ammonium and cerium nitrate R*, add five successive quantities, each of 100 mL, of *water R*, shaking after each addition. Dilute the clear solution to 1000.0 mL with *water R*. Standardise the solution after 10 days.

*Standardisation*. To 25.0 mL of the ammonium and cerium nitrate solution add 2.0 g of *potassium iodide R* and 150 mL of *water R*. Titrate immediately with 0.1 M *sodium thiosulfate*, using 1 mL of *starch solution R* as indicator.

*Storage*: protected from light.

#### 0.01 M Ammonium and cerium nitrate. 3000200.

To 100.0 mL of 0.1 M *ammonium and cerium nitrate* add, with cooling, 30 mL of sulfuric acid R and dilute to 1000.0 mL with *water R*.

#### 0.1 M Ammonium and cerium sulfate. 3000300.

Dissolve 65.0 g of *ammonium and cerium sulfate R* in a mixture of 500 mL of *water R* and 30 mL of sulfuric acid R. Allow to cool and dilute to 1000.0 mL with *water R*.

*Standardisation*. To 25.0 mL of the ammonium and cerium sulfate solution add 2.0 g of *potassium iodide R* and 150 mL of *water R*. Titrate immediately with 0.1 M *sodium thiosulfate*, using 1 mL of *starch solution R* as indicator.

#### 0.01 M Ammonium and cerium sulfate. 3000400.

To 100.0 mL of 0.1 M *ammonium and cerium sulfate* add, with cooling, 30 mL of sulfuric acid R and dilute to 1000.0 mL with *water R*.

#### 0.1 M Ammonium thiocyanate. 3000500.

Dissolve 7.612 g of *ammonium thiocyanate R* in *water R* and dilute to 1000.0 mL with the same solvent.

*Standardisation*. To 20.0 mL of 0.1 M *silver nitrate* add 25 mL of *water R*, 2 mL of *dilute nitric acid R* and 2 mL of *ferric ammonium sulfate solution R2*. Titrate with the ammonium thiocyanate solution until a reddish-yellow colour is obtained.

#### 0.1 M Barium chloride. 3000600.

Dissolve 24.4 g of *barium chloride R* in *water R* and dilute to 1000.0 mL with the same solvent.

*Standardisation*. To 10.0 mL of the barium chloride solution add 60 mL of *water R*, 3 mL of *concentrated ammonia R* and 0.5-1 mg of *phthalein purple R*. Titrate with 0.1 M *sodium edetate*. When the solution begins to decolorise, add 50 mL of *ethanol (96 per cent) R* and continue the titration until the blue-violet colour disappears.