AMALTHEA

PROTOCOLS

COMMONLY USED REAGENTS RECIPES

iGEM THESSALY

Commonly Used Reagents Recipes

Acid precipitation solution

- 1 M HCl
- 0.1 M sodium pyrophosphate

Nucleic acids can also be precipitated with a 10% (w/v) solution of trichloroacetic acid (TCA); however, this recipe is cheaper, easier to prepare, and just as efficient.

Ammonium acetate, 10 M

- Dissolve 385.4 g ammonium acetate in 150 ml H2O
- Add H2O to 500 ml

BBS (BES-buffered solution), 2×

- 50 mM N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES; Calbiochem)
- 280 mM NaCl
- 1.5 mM Na2HPO4, pH 6.95
- 800 ml H2O
- Adjust pH to 6.95 with room temperature 1 M NaOH
- H2O to 1 liter
  Filter sterilize through a 0.45-μm nitrocellulose filter (Nalgene)
  Store in aliquots at −20°C (can be frozen and thawed repeatedly)
  The pH of this solution is critical (pH 6.95 to 6.98). When a new batch of 2× BES buffer is prepared, its pH should be checked against a reference stock prepared (and tested) earlier.

CaCl2 , 1 M

- 147 g CaCl2·2H2O
- H2O to 1 liter

Denhardt solution, 100×

- 10 g Ficoll 400
- 10 g polyvinylpyrrolidone
- 10 g bovine serum albumin (Pentax Fraction V; Miles Laboratories)
- H2O to 500 ml

Filter sterilize and store at −20°C in 25-ml aliquots

Dithiothreitol (DTT), 1 M

- Dissolve 15.45 g DTT in 100 ml H2O
  Store at −20°C

EDTA (ethylenediamine tetraacetic acid), 0.5 M (pH 8.0)

- Dissolve 186.1 g Na2EDTA·2H2O in 700 ml H2O
- Adjust pH to 8.0 with 10 M NaOH (~50 ml)
- Add H2O to 1 liter

HBSS (Hanks balanced salt solution)

- 5.4 mM KCl
- 0.3 mM Na2HPO4
- 0.4 mM KH2PO4
- 4.2 mM NaHCO3
- 1.3 mM CaCl2
- 0.5 mM MgCl2
- 0.6 mM MgSO4
- 137 mM NaCl
- 5.6 mM D-glucose
- 0.02% phenol red (optional)
- Add H2O to l liter and adjust pH to 7.4

HBSS can be purchased from Biofluids or Whittaker.
HBSS may be made or purchased without CaCl2 and MgCl2. These are optional components that usually have no effect on an experiment. In some cases, however, their presence may be detrimental to a procedure. Consult the individual protocol to see if the presence or absence of these components is recommended in the materials list.

HCl, 1 M

Mix in the following order:

- 913.8 ml H2O
- 86.2 ml concentrated HCl

HeBS (HEPES-buffered saline) solution, 2×

- 16.4 g NaCl
- 11.9 g HEPES acid
- 0.21 g Na2HPO4
- 800 ml H2O
- Titrate to pH 7.05 with 5 M NaOH
- Add H2O to 1 liter

Filter sterilize through a 0.45-µm nitrocellulose filter

Test for transfection efficiency and store at −20°C in 50-ml aliquots

An exact pH is extremely important for efficient transfection. The optimal pH range is 7.05 to 7.12.

KCl, 1 M

- 74.6 g KCl
- H2O to 1 liter

MgCl2, 1 M

- 20.3 g MgCl2·6H2O
- H2O to 100 ml

MgSO4 , 1 M

- 24.6 g MgSO4·7H2O
- H2O to 100 ml

MOPS buffer

- 0.2 M MOPS [3-(N-morpholino)propanesulfonic acid], pH 7.0
- 0.5 M sodium acetate
- 0.1 M EDTA

Store in the dark and discard if it turns yellow.

NaCl, 5 M

- 292 g NaCl
- H2O to 1 liter

NaOH, 10 M

- Dissolve 400 g NaOH in 450 ml H2O
- Add H2O to 1 liter

PBS (phosphate-buffered saline)

10× stock solution, 1 liter:

- 80 g NaCl
- 2 g KCl
- 11.5 g Na2HPO4·7H2O
- 2 g KH2PO4

Working solution, pH ∼7.3:

- 137 mM NaCl
- 2.7 mM KCl
- 4.3 mM Na2HPO4·7H2O
- 1.4 mM KH2PO4

Potassium acetate buffer, 0.1 M

- Solution A: 11.55 ml glacial acetic acid/liter (0.2 M).
- Solution B: 19.6 g potassium acetate (KC2H3O2)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H2O to 100 ml. *This may be made as a 5- or 10-fold concentrate by scaling up the amount of potassium acetate in the same volume. Acetate buffers show concentration-dependent pH changes, so check concentrate pH by diluting an aliquot to the final concentration. To prepare buffers with pH intermediate between the points listed in Table A.2.2, prepare closest higher pH, then titrate with solution A.*

Table A.2.2 Preparation of 0.1 M Sodium and Potassium Acetate Buffers[a]

Desired pH	Solution A (ml)	Solution B (ml)
3.6	46.3	3.7
3.8	44.0	6.0
4.0	41.0	9.0
4.2	36.8	13.2
4.4	30.5	19.5
4.6	25.5	24.5
4.8	20.0	30.0
5.0	14.8	35.2
5.2	10.5	39.5
5.4	8.8	41.2
5.6	4.8	45.2

[a]Adapted by permission from CRC (1975).

Potassium phosphate buffer, 0.1 M

- Solution A: 27.2 g KH2PO4 per liter (0.2 M).
- Solution B: 34.8 g K2HPO4 per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H2O to 200 ml. *This may be made as a 5- or 10-fold concentrate by scaling up the amount of potassium phosphate in the same volume. Phosphate buffers show concentration-dependent pH changes, so check concentrate pH by diluting an aliquot to the final concentration*.

Table A.2.3 Preparation of 0.1 M Sodium and Potassium Phosphate Buffers[a]

Desired pH	Solution A (ml)	Solution B (ml)	Desired pH	Solution A (ml)	Solution B (ml)
5.7	93.5	6.5	6.9	45.0	55.0
5.8	92.0	8.0	7.0	39.0	61.0
5.9	90.0	10.0	7.1	33.0	67.0
6.0	87.7	12.3	7.2	28.0	72.0
6.1	85.0	15.0	7.3	23.0	77.0
6.2	81.5	18.5	7.4	19.0	81.0
6.3	77.5	22.5	7.5	16.0	84.0
6.4	73.5	26.5	7.6	13.0	87.0
6.5	68.5	31.5	7.7	10.5	90.5
6.6	62.5	37.5	7.8	8.5	91.5
6.7	56.5	43.5	7.9	7.0	93.0
6.8	51.0	49.0	8.0	5.3	94.7

[a]Adapted by permission from CRC (1975).

SDS electrophoresis buffer, 5×

- 15.1 g Tris base
- 72.0 g glycine
- 5.0 g SDS
- H2O to 1000 ml
- Dilute to 1× or 2× for working solution, as appropriate

Store up to 1 month at 0° to 4°C
Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted.

SED (standard enzyme diluent)

- 20 mM Tris·Cl, pH 7.5
- 500 µg/ml bovine serum albumin (Pentax Fraction V)
- 10 mM 2-mercaptoethanol

Store up to 1 month at 4°C

Sodium acetate, 3 M

- Dissolve 408 g sodium acetate·3H2O in 800 ml H2O
- Add H2O to 1 liter

Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2 M).

Solution B: 27.2 g sodium acetate (NaC2H3O2·3H2O)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H2O to 100 ml. (See Potassium acetate buffer recipe for further details.)

Sodium phosphate buffer, 0.1 M

- Solution A: 27.6 g NaH2PO4·H2O per liter (0.2 M).
- Solution B: 53.65 g Na2HPO4·7H2O per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H2O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

SSC (sodium chloride/sodium citrate), 20×

- 3 M NaCl (175 g/liter)
- 0.3 M Na3citrate·2H2O (88 g/liter)
- Adjust pH to 7.0 with 1 M HCl

STE buffer

- 10 mM Tris·Cl, pH 7.5
- 10 mM NaCl
- 1 mM EDTA, pH 8.0

TE (Tris/EDTA) buffer

- 10 mM Tris·Cl, pH 7.4, 7.5, or 8.0 (or other pH; see recipe)
- 1 mM EDTA, pH 8.0

TEA (triethanolamine) solution

- 50 mM triethanolamine, pH ~11.5
- 0.1% Triton X-100
- 0.15 M NaCl

Add Triton X-100 as a 10% stock sterilized by Millipore filtration and stored in the dark to prevent photooxidation (stock is stable 5 years at room temperature).

TEN (Tris/EDTA/NaCl) solution

- 40 mM Tris·Cl, pH 7.5
- 1 mM EDTA, pH 8.0
- 150 mM NaCl

Store up to 6 months at room temperature

TM buffer, 10×

- 100 mM Tris·Cl, pH 8.0
- 100 mM MgCl2

Tris-buffered saline (TBS)

- 100 mM Tris·Cl, pH 7.5
- 0.9% (150 mM) NaCl

Store up to several months at 4°C

Tris·Cl [tris(hydroxymethyl)aminomethane], 1 M

- Dissolve 121 g Tris base in 800 ml H2O
- Adjust to desired pH with concentrated HCl
- Mix and add H2O to 1 liter

Approximately 70 ml of HCl is needed to achieve a pH 7.4 solution, and approximately 42 ml for a solution that is pH 8.0.

IMPORTANT NOTE: *The pH of Tris buffers changes significantly with temperature, decreasing approximately 0.028 pH units per 1°C. Tris-buffered solutions should be adjusted to the desired pH at the temperature at which they will be used. Because the pKa of Tris is 8.08, Tris should not be used as a buffer below pH ~7.2 or above pH ~9.0.*

TTBS (Tween 20/TBS)

- 0.1% Tween 20 in Tris-buffered saline (TBS; see recipe)

Store up to several months at 4°C

1 M IPTG (1 M IPTG Recipe) Stock

1. Dissolve 2.38 g of IPTG in 8 mL of distilled H2O.
2. Bring to a final volume of 10 mL with molecular biology grade H2O.
3. Filter sterilize with a 0.22 µ syringe filter.
4. Store in 1mL aliquots at -20 °C.

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