General-purpose solutions

ABBREVIATIONS: DW de-ionized water.

LABELLING. Adhesive tape must be used. The following solutions may be labelled with the specified abbreviation — preferably using a bold felt-tip pen, together with date prepared and initials of person who prepared it.

Trypanosome Dilution Buffer (TDB: Paul Voorheis)

	5 x concentrate	1x working solution
0.005 M KCl	1.86 g/1	0.37 g/1
0.080 NaCl	23.4	4.68
0.001 $MgSO_4.7H_20$	1.24	0.25
0.020 Na_2HPO_4	14.2	2.84
0.002 $NaH_2PO_4.2H_20$	1.55	0.31
0.020 glucose	18.0	3.60

Dissolve $MgSO_4.7H_2O$ separately and add slowly, with stirring, once the other components have been dissolved and made up almost to final volume. Check pH ONLY AFTER IT IS DILUTED TO 1X — it should be 7.7 and NOT need adjustment! If the pH is wrong this indicates that components were incorrect or misweighed, etc). Bottle 5x concentrate in exactly 50 ml amounts; 1x working solution in 100ml amounts and store in freezer.

Working Solution from 5x concentrate Thaw concentrate and warm to around 50°. Mix and check everything has re-dissolved. Make up to 250 ml using DW. Check pH. Transfer to 250 ml bottle. Date, initial and store in fridge. Prepare freshly each week. This solution may also be required sterile (filter sterilize, then store at 20°).

Citrate-Glucose Anticoagulant (CGA)

0.1 M Trisodium citrate.$2H_2O$	30 g/l
0.040 M glucose	7.2 g

The pH of this has to be adjusted to 7.7 with NaOH. Freeze in 100 ml amounts. Initial and date. Re-date when thawed and transferred to fridge. Discard after 2 weeks in fridge.

Breaking Buffer (BB)

0.050 M Tris	6.02 g/l
0.005 M Magnesium acetate	1.07
0.050 M KCl	3.73

Make up in DW and adjust pH to 7.8 with NaOH.

Separation Buffer (Phosphate-buffered-Saline-Glucose: PSG)

(modified 11/76 — substituting KH_2PO_4 for NaH_2PO_4).

44 mM NaCl, 57 mM Na_2HPO_4, 3 mM KH_2PO_4, 55 mM glucose

Stock Solutions —

1,000 ml 0.4 M Na_2HPO_4 (anhydrous) 56.8 g/l

100 ml 0.2 M KH_2PO_4 (anhydrous) 2.72 g/100 ml

1,000 ml 1.5 M NaCl 87.7 g/l.

These solutions should be stored at 20° in good quality clear glass bottles. Check for absence of crystallization, due to low temperature, or other deterioration before use.

Working Solution —

143 ml 0.4 M Na_2HPO_4

15.0 ml 0.2 M KH_2PO_4

30.0 ml 1.5 M NaCl

10.0 g glucose

Make up to 1 litre with DW and check pH is 8.0 at 20 °C. IT SHOULD NOT NEED ADJUSTING AND MUST NOT BE ADJUSTED. If pH is off, it means the solutions were not prepared accurately. This comment applies to all phosphate buffered solutions! The working solution should be prepared weekly from stock solutions and stored at 4°C.

ALTERNATIVELY, prepare 1 liter of working solution directly as follows—

8.12 g Na_2HPO_4 (anhydrous)

0.41 g KH_2PO_4 (anhydrous)
2.63 g NaCl
10 g glucose

Make up to 1 liter and check pH is 8.0 at 20°C (it will NOT need any adjustment if ingredients are correctly weighed). Store at 4°C.

ALTERNATIVELY, prepare 1 liter of 10x solution minus glucose as follows—

81.2 g Na_2HPO_4 (anhydrous) (final concentration 0.57 M)

4.1 g KH_2PO_4 (anhydrous) (final concentration 0.030 M)

26.3 g NaCl (final concentration 0.44 M)

Store at room temperature.

This can be diluted 10x for regular use or 2x for initial DE52 equilibration purposes. Check pH only after diluting.

Bicine-buffered saline glucose (BSG)

(Taylor, Lanham & Williams, 1974)
Per liter of working-strength solution (pH 8.0)

8.16 g bicine (0.050 M)

1.26 g glucose (0.007 M)

0.372 g KCl (0.005 M)

0.813 g $MgCl_2$ (0.0085 M)

2.92 g NaCl (0.050 M)

18.83 g sucrose (0.055 M)

100 ml 0.2M $CaCl_2$ (22.2 g/l of anhydrous $CaCl_2$)

35 ml 1.0M NaOH. (40 g/l)
Check the pH and store at 4°C — but not for long!

BBSG (Jayne Raper)

Per liter of working solution

8.16 g Bicine (0.050 M)

12.6 g glucose (0.077 M: yes, really)

2.92 g NaCl (0.050 M)

0.372 g KCl (0.005 M)

pH to 8.0 with NaOH (should need about 35 ml of 1.0 M NaOH)

BBSG is based on Taylor, Lanham & Williams BSG (see above), but is much simpler. Jayne's comments: BBSG is isotonic. When you add to DEAE-52 recalibrate the pH [George's comment: this should not be necessary if the DEAE was properly equilibrated]. Store DEAE-52 without glucose (BBS). And then wash the column with two column volumes of BBSG before use. If you are isolating tryps to study a surface protein, add 5% BSA to the BBSG (George's commnet: presumably not the buffer to be used on the column) recheck the pH, make sure it is 8.0.

I decided to make the first batch (11/4/3) adding 5 mM Ca, as a compromise with 20 mM in BSG, because some Ca is probably good for cell stability).

BBCSG (George)

Per liter of working solution

8.16 g Bicine (0.050 M)

12.6 g glucose (0.077 M: a really high concentration!)

2.92 g NaCl (0.050 M)

0.372 g KCl (0.005 M)

0.56 g $CaCl_2$ (0.005 M)

pH to 8.0 with NaOH (should need about 35 ml of 1.0 M NaOH)