Preparation of 2 liters of 10x concentrated PBS stock solution (phosphate buffered saline)

Intended use: After 1:10 dilution of the PBS 10x stock solution with distilled water, PBS is used to equilibrate the DEAE gel and as buffer for PSG preparation.

Storage: The PBS 10x stock solution can be kept at room temperature for one week.

Safety: Reagents are not toxic, explosive or corrosive.

Material

Distiller
Weighing cup
Balance with 10-mg precision
Magnetic stirrer with magnets (balance and stirrer on separate benches)
pH meter
Conductimeter
2000-ml beaker
2000-ml flask
Parafilm
Spatula or spoon
1000-ml plastic bottle
Scissors
Indelible marker
Funnel

Reagents

Distilled water, >2 liters
Di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$)
Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$)
Sodium chloride (NaCl)
Standard buffers for pH meter: citrate/hydrochloric acid pH 4.0, phosphate pH 7.0

Composition

$Na_2HPO_4.2H_2O$	75,0 g/l
$NaH_2PO_4.H_2O$	3,45 g/l
NaCl	21,25 g/l

pH: between 7.7 and 7.8

Procedure

1. Control that all materials and reagents are available and clean.
2. Pour 1000 ml of water into a clean 2000-ml beaker.
3. Add a clean magnetic stirrer.
4. Put the beaker on a stirrer. Turn stirrer on.
5. Weigh the reagents, write down the measured weights on the production sheet and add the reagents into the beaker.
6. Keep stirring until full dissolution of crystals (at least 30 minutes).
7. Transfer into a 2000-ml flask.
8. Adjust volume with distilled water up to 2000 ml.
9. Calibrate the pH meter **(see pH meter manual).**
10. Measure the pH, which should be between 7.7 and 7.85.
11. Close the flask with parafilm. Write the date, pH and name of the person who prepared the solution on the flask.
12. Store at room temperature (do not store at 4°C!!).

PRODUCTION SHEET

Preparation of 2 liters of 10x concentrated PBS stock solution (phosphate buffered saline)

DATE	
PERSON WHO PREPARED THE SOLUTION	
CALIBRATE pH METER	

REAGENT	**MANUFACTURER**	**BATC**	**EXPECTED**	**MESURED**
$NA_2HPO_4.2H_2O$			**150,50 g**	
$NAH_2PO_4.H_2O$			**6,90 g**	
NaCl			**42,50 g**	

pH	**7.70 - 7.85**	

Preparation of 10 liters of PBS buffer for gel equilibration

Intended use: PBS buffer is used to equilibrate the gel. 10 liters are sufficient to equilibrate 1 kg of gel.

Storage: PBS buffer can be kept at 4°C for one week.

Safety: Reagents are not toxic, explosive or corrosive.

Equipment

1000-ml cylinder
pH meter
Conductimeter
1000-ml plastic bottle
Waterproof marker
Funnel
Clean 10 or 20-liter container

Reagents

9 liters of distilled water
1 liter of PBS 10x stock solution
Standard solution for conductimeter
Standard buffers for pH meter: citrate/hydrochloric acid pH 4.0, phosphate pH 7.0

Composition

$Na_2HPO_4.2H_2O$ 7.5 g/l
$NaH_2PO_4.H_2O$ 0.34 g/l
NaCl 2.12 g/l
pH: between 7.9 and 8.1

NOTE

The units used to express conductivity are mmho/cm or milli-Siemens/cm

x mmho/cm = x milli-Siemens/cm

Procedure

1. Control that all materials and reagents are available and clean.
2. Using a 1000-ml cylinder, measure 1000 ml of PBS 10x stock solution (see SOP M/1).
3. Transfer into a clean 10-liter container.
4. Add 9 x 1000 ml of distilled water (measured with the 1000-ml cylinder).
5. Close the container and mix the solution by shaking vigorously.
6. Calibrate the pH meter **(see pH meter manual).**
7. Take an aliquot of the PBS buffer and measure its pH (pH between 8.0 and 8.1).
8. Calibrate the conductimeter **(see conductimeter manual).**
9. Record the temperature and conductivity of the PBS buffer (conductivity ~9 mmho/cm or mS/cm).
10. Close the container. Write the date, pH, temperature, conductivity and name of the person who prepared the solution on the container.
11. Use the buffer for a few days only.

PRODUCTION SHEET

Preparation of 10 liters of PBS buffer (phosphate buffered saline)

DATE	
PERSON WHO PREPARED THE SOLUTION	
CALIBRATE pH METER	
CALIBRATE CONDUCTIMETER	

	EXPECTED pH	**MEASURED pH**
MEASURE PBS BUFFER pH	**7.90 - 8.10**	

	TEMP. °C	**EXPECTED CONDUCTIVITY**	**MEASURED CONDUCTIVITY**
MEASURE PBS BUFFER TEMPERATURE AND CONDUCTIVITY		**9 mS/cm**	

Preparation of 15 liters of PSG buffer for gel equilibration

Intended use: PSG buffer is used for final gel equilibration during mounting of the minicolumns.

Storage: PSG buffer should be prepared the day of use and should NOT be stored.

Safety: Reagents are not toxic, explosive or corrosive.

Equipment

5000-ml flask
1000-ml cylinder
Parafilm
Magnetic stirrer with magnets
pH meter
Conductimeter
1000-ml plastic bottle
Scissors
Waterproof marker
Funnel
Precision balance
0.22-µm filter on disposable bottle
Manual vacuum pump
2 very clean 20-liters polycarbonate containers, preferably autoclaved

Reagents

13,5 liters of distilled water
1.5 liters of PBS 10x stock solution
Anhydrous D-glucose
Standard solution for conductimeter
Standard buffers for pH meter: citrate/hydrochloric acid pH 4.0, phosphate pH 7.0

Composition

$Na_2HPO_4.2H_2O$	7.5 g/l
$NaH_2PO_4.H_2O$	0.34 g/l
NaCl	2.12 g/l
Anhydrous D-glucose	10 g/l

pH: between 7.9 and 8.1

NOTE

The units used to express conductivity are mmho/cm or milli-Siemens/cm

x mmho/cm = x milli-Siemens/cm

Procedure

1. Control that all materials and reagents are available and clean.
2. Prepare 1.5 litres of PBS stock solution buffer (PBS 10x, see SOP M/1)
3. Transfer these 1.5 liters into a 5000-ml flask.
4. Weigh 150 g of anhydrous glucose and add them into the 5000-ml flask.
5. Add distilled water up to the 5000-ml mark.
6. Add a magnetic stirrer and mix for 30 minutes.
7. Transfer the buffer into a clean 20-liter polycarbonate container.
8. Measure 5000 ml of distilled water using the 5000-ml flask and add it to the container.
9. Repeat this step once to obtain 15 liters of PSG buffer
10. Close the container and mix the solution by shaking vigorously.
11. Calibrate the pH meter **(see pH meter manual).**
12. Take a PSG buffer aliquot and measure its pH (pH between 8.0 and 8.1).
13. Calibrate the conductimeter **(see conductimeter manual).**
14. Record the temperature and conductivity of the PSG buffer (conductivity ~9 mmho/cm or mS/cm).
15. Pass the buffer through the 0.22-µm filter into a new, very clean 20-liters polycarbonate container.
16. Close the container. Write pH, temperature, conductivity and name of the person who prepared the solution.
17. Use within 2 days.
18. Discard any remaining PSG, rinse the containers with distilled water, autoclave if possible, and keep them closed until next use.

PRODUCTION SHEET

Preparation of 15 liters of PSG buffer (Phosphate saline glucose)

DATE	
PERSON WHO PREPARED THE SOLUTION	
CALIBRATE pH METER	
CALIBRATE CONDUCTIMETER	

REAGENT	**MANUFACTURER**	**BATCH**	**EXPECTED**	**MESURED**
GLUCOSE			**200,0 g**	

	EXPECTED pH	**MEASURED pH**
MEASURE PSG BUFFER pH	**7.90 - 8.10**	

	TEMP. °C	**EXPECTED CONDUCTIVITY**	**MEASURED CONDUCTIVITY**
MEASURE PSG BUFFER TEMPERATURE AND CONDUCTIVITY		**9 mS/cm**	

Previous version: November 2008
Changes to previous version:

- **Equipment**
  2 very clean 20-liters polycarbonate containers, preferably autoclaved
- **Procedure**
  Transfer the buffer into a clean 20-liter polycarbonate container.
  Pass the buffer through the 0.22-µm filter into a new, very clean 20-liters polycarbonate container.
  Discard any remaining PSG, rinse the polycarbonate containers with distilled water, autoclave if possible, and keep them closed until next use.

Previous version: October 2007
Changes to previous version:
Preparation adapted for 15 liters

- **Reagents**
  13.5 liters of distilled water
- **Procedure:**
  9. Repeat this step once to obtain 15 liters of PSG buffer
  17. Use within 2 days

Previous version: June 2007
Changes to previous version:

- **Storage:** PSG buffer should be prepared the day of use and should NOT be stored.
- **Procedure:**
  17. Use the same day
  18. Discard any remaining PSG, rinse the container with distilled water and keep it closed until next use.

Preparation of a diluted (20 x) phosphoric acid solution

Intended use: The diluted phosphoric acid solution is used to bring the pH of the wet gel to 8.

Storage: The diluted phosphoric acid solution can be kept at room temperature for one month.

Safety: Toxic and corrosive reagent to handle under a hood. Do not inhale. Protect your eyes. In the event of an accident, rinse abundantly with water.

Equipment

Hood
100-ml cylinder
Single-use transfer pipette
500-ml flask
Funnel
Parafilm
Scissors
1000-ml Schott bottle
Waterproof marker

Reagents

100 ml of distilled water
Orthophosphoric acid (85%, d: 1.71)

Final concentration

H_3PO_4 4.25 %

Procedure

1. Control that all materials and reagents are available and clean.
2. Under the hood, measure 25 ml of phosphoric acid in the 10-ml cylinder.
3. Transfer into the 500-ml flask.
4. Add distilled water up to the 500-ml mark.
5. Cap the flask with parafilm and mix thoroughly.
6. Transfer into a 1000-ml Schott bottle.
7. Write the date, content (phosphoric acid 20 x) and name of the person who prepared the solution.
8. Store at room temperature.

PRODUCTION SHEET

Preparation of a diluted (20 x) phosphoric acid solution

DATE	
PERSON WHO PREPARED THE SOLUTION	

REAGENT	**MANUFACTURER**	**BATCH**
H_3PO_4		

	MEASURED VOLUME (ML)
MEASURE VOLUME OF CONCENTRATED ACID	
MEASURE VOLUME OF WATER	
TOTAL VOLUME OF DILUTED (20X) PHOSPHORIC ACID	

Previous version June 2007
Changes:

- **Storage:** The diluted phosphoric acid solution can be kept at room temperature for one month.

Preparation of 1900-ml DEAE gel equilibrated with PSG buffer Volume sufficient for 700 - 750 minicolumns

Intended use: The equilibrated DEAE gel is used to fill the minicolumns. Six volumes of gel allow the separation of the *Trypanozoon* group (*T. brucei, T. evansi, T. equiperdum*) trypanosomes from one volume of human, rat or mouse blood.

Storage: A few days at 4°C. For longer storage, keep at –20 °C.

Safety: Diluted orthophosphoric acid: corrosive

Material:

Balance with 100-mg precision
Weighing cup
Parafilm
Scissors
Magnetic stirrer with magnets
pH meter
Conductimeter
Plastic stick
Transfer pipettes
5000-ml glass beaker
2 plastic graduated beakers of 2000 ml
Spatula or spoon
Waterproof marker

Reagents

DEAE-cellulose (Diethylaminoethyl–cellulose) gel: Whatman DE52
Diluted (20x) orthophosphoric acid (see SOP M/4)
PBS buffer (SOP M/2), about 10 liters
PSG buffer (SOP M/3), about 6 liters
Standard buffers for pH meter
Standard solution for conductimeter

Composition

40% DEAE–cellulose suspension in PSG buffer
pH: between 7.90 and 8.10
Conductivity: 9 mmho/cm ormS/cm

Procedure (10 liters of PBS)

1. Control that all materials and reagents are available and clean.
2. Take the PBS buffer out of the fridge and allow it to equilibrate to room temperature.
3. Weigh 1000 g of DEAE cellulose and transfer it into a clean 5-liter beaker.
4. Add a clean stirring magnet.

Equilibration with PBS followed by PSG

5. Add 3000 ml of PBS, whose pH (X), conductivity (Y) and temperature (Z) have been noted on the production sheet.
6. Put the beaker on a stirrer. Mix for 10 min.
7. While mixing, bring to pH 8.0 by adding diluted orthophosphoric acid with a transfer pipette and note the volume of added orthophosphoric acid (approximately 125 ml).
8. Let the gel sediment (45 min) and decant.
9. *First wash*: Add 3000 ml of PBS and mix on the stirrer for 10 min. Let the gel sediment (45 min) and decant. Take an aliquot of supernatant and control the pH, conductivity and temperature of the supernatant and of the PBS buffer.
10. *Second wash*: Add 3000 ml of PBS and mix for 10 min. Let the gel sediment (45 min) and decant. Take an aliquot of supernatant, and control the pH, conductivity and temperature of the supernatant and of the PBS buffer.
11. *Third wash*: Add 3000 ml of PSG and mix for 10 min. Let the gel sediment (45 min) and decant. Take an aliquot of supernatant, and control the pH, conductivity and temperature of the supernatant and of the PSG buffer.
12. *Final equilibration*: Add 3000 ml of PSG and mix well.

Equilibrated gel volume measurement

13. Transfer the well mixed gel suspension into three graduated 2000 ml plastic beakers and let sediment overnight at ambient temperature
14. The next morning, measure the volume of the sedimented gel in the beakers (total is approximately 1900 ml).
15. Without mixing the sediment, take the supernatant leaving a supernatant volume 1,5 x the volume of the sedimented gel (total volume around 4,75 liters, sufficient to fill 700 to 750 minicolumns).

PRODUCTION SHEET

Preparation of 1900-ml DEAE gel equilibrated with PSG buffer

DATE	
PERSON WHO PREPARED THE GEL	
CALIBRATE pH METER	
CALIBRATE CONDUCTIMETER	

REAGENT	**LOT**	**EXPECTED**	**MEASURED**
WHATMAN DEAE CELLULOSE		**1000 g**	

PRODUCTION SHEET

Preparation of 1900-ml DEAE gel equilibrated with PSG buffer

DATE	
PERSON WHO PREPARED THE GEL	

	EXPECTED	MEASURED
PBS pH	**(X)**	
PBS conductivity (9 mmho/cm or mS/cm)	**(Y)**	
PBS temperature (°C)	**(Z)**	
pH of gel with PBS	**10 - 11**	
volume of PBS added (ml)	**3000**	
volume of phosphoric acid added 1/20 (ml)	**125**	
gel pH after phosphoric acid addition	**8.0**	

FIRST WASH	EXPECTED		MEASURED
volume of PBS added (ml)	**3000**		
supernatant pH	**8**	**X**	
supernatant conductivity (9 mmho/cm or mS/cm)	**9**	**Y**	
supernatant temperature (°C)	**---**	**Z**	

SECOND WASH	EXPECTED		MEASURED
volume of PBS added (ml)	**3000**		
supernatant pH	**8**	**X**	
supernatant conductivity (9 mmho/cm or mS/cm)	**9**	**Y**	
supernatant temperature (°C)		**Z**	

	EXPECTED	MEASURED
PSG pH	**(X)**	
PSG conductivity (9 mmho/cm or mS/cm)	**(Y)**	
PSG temperature (°C)	**(Z)**	

THIRD WASH	EXPECTED		MEASURED
volume of PSG added (ml)	**3000**		
supernatant pH	**8**	**X**	
supernatant conductivity (9 mmho/cm or mS/cm)	**9**	**Y**	
supernatant temperature (°C)	**---**	**Z**	

FINAL EQUILIBRATION	EXPECTED	MEASURED
volume of PSG added (ml)	**3000**	

VOLUME OF EQUILIBRATED GEL	EXPECTED	MEASURED
FIRST BEAKER	**633,33**	
SECOND BEAKER	**633,33**	
THIRD BEAKER	**633,33**	
TOTAL VOLUME OF SEDIMENTED GEL (ML)	**1900**	

Previous version: November 2008

Changes:

Page 2
Step 13 in process: change TWO into THREE beakers.

Page 5
Table Final Equilibration: omit 3 last rows

FINAL EQUILIBRATION	EXPECTED		MEASURED
volume of PSG added (ml)	3000		
~~supernatant pH~~	~~8~~	~~X~~	
~~supernatant conductivity (9 mmho/cm or mS/cm)~~	~~9~~	~~Y~~	
~~supernatant temperature (°C)~~		~~Z~~	

Page 5
Table Volume of equilibrated gel
Add one row and indicate expected volumes

VOLUME OF EQUILIBRATED GEL	EXPECTED	MEASURED
FIRST BEAKER	633,33	
SECOND BEAKER	633,33	
THIRD BEAKER	633,33	
TOTAL VOLUME OF SEDIMENTED GEL (ML)	1900	