PROVIDING SOLUTIONS:

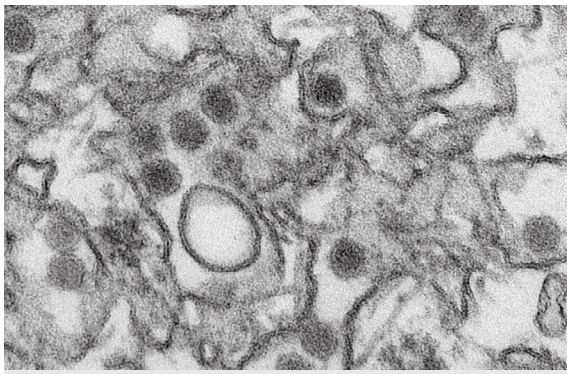
Buffers for Electron Microscopy

KIRSCH NOTES
PROTOCOLS & TIPS FOR MICROSCOPY

There are seven common buffers used for biological sample preparation for TEM: *Phosphate (Sorensen's Phosphate, Millonig's Phosphate), Cacodylate, PHEM, S-Collidine, Tris, Hepes, and PIPES.*

There are three primary functions for the use of buffers in biological sample preparation:

1. The ability to maintain a constant pH (7.2 – 7.4 for mammalian and 6.9 for plants) during fixation **(NOTE: The reaction between proteins and non-buffered aldehydes results in a drastic lowering of pH and the generation of morphologic artifacts.)**
2. To provide a vehicle for the fixative which contributes to the necessary osmolarity of the solution, which should be slightly hypertonic.
3. To contain compatible ionic moieties to avoid extraction or precipitation of constituent ions.



Transmission Electron micrograph of Zika Virus. Virus particles are 40 nm in diameter, with an outer envelope and an inner dense core. Courtesy of Cynthia Goldsmith, CD.

Phosphate Buffers

Phosphate Buffer is an excellent buffer with several specific recipes, physiologically compatible with cells, and is non-toxic. It can be adapted to function optimally at a variety of pH levels by altering the ratios of the monobasic and the dibasic compounds. Typically, for pH 7.3, a 1:2.3 mono-basic : di-basic ratio is used, with more monobasic for lower or more dibasic for higher pH ranges. The osmolarity is sometimes augmented by the addition of sucrose or sodium chloride.

The addition of sucrose can support growths when stored for many months, even at 4˚C. Calcium cannot be used with phosphate buffer since it is precipitated with citrates.

Sorensen's Phosphate
Mono and dibasic phosphates with 0.18M sucrose added. The osmolarity of the 0.1M, pH 7.2 solution is 425. Shelf life is approximately 3 months.

Millonig's Phosphate
The typical combination of mono and dibasic phosphates, with the addition of 0.5% sodium chloride. This buffer is hypertonic (pH 7.4 - 0.1M - 440mosmols) and is recommended for very hydrated tissues. **NOTE: For marine organisms use 3% NaCl.**

Advantages	Disadvantages
1. It is non-toxic	**1.** It is incompatible with calcium ions.
2. Has no special disposal requirements (it can be poured down the drain).	
3. Can be custom tailored to function optimally at a variety of pH ranges.	
4. Minimal pH change with temperature	
5. Components and pre-prepared solutions are low in cost.	

Cacodylate Buffers

Cacodylate Buffer is a buffer that maintains pH levels very well during fixation. This buffer contains arsenic and is potentially carcinogenic. These properties require it to be collected and disposed of according to state and federal guide lines.

Advantages	Disadvantages
1. Suitable for use with calcium, does not form precipitates	**1.** Potential carcinogen and contains arsenic, controlled disposal required
2. Long shelf life, does not support growths	**2.** Expensive

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PHEM Buffer

PHEM buffer has been used primarily for tissues and cell cultures being processed for immunocytochemical studies. Most antigens, especially intra cellular ones, stain better using PHEM than those processed using PBS, it also has a more limited effect on biochemical reactions and enzymes. Very good preservation of cellular ultrastructure, notably microtubules, is also an advantage of PHEM use.

Advantages

1. Very good preservation of cellular ultrastructure
2. Limited effect on biochemical reactions and enzymes

S-Collidine Buffer

A very stable buffer typically used to buffer OsO_4 providing excellent fixation. A very strong odor and the presence of pyrimidine makes it very toxic and difficult to work with.

Advantages

1. Excellent for buffering osmium tetroxide in 2nd fixation
2. Very good stability and buffering capacity – max. pH 7.4

Disadvantages

1. Very strong odor, requires fume hood
2. Toxic pyrimidine component
3. Not useful with aldehyde primary fixative

Tris, Tris Maleate Buffers

Advantages

1. Functional at high pH and temperature ranges used for antigen retrieval
2. Improves antibody accessibility for immuno histo/cyto chemistry

Disadvantages

1. Large pH change with temperature, must be adjusted at used temperature

Cell Culture Buffers

Hepes (Good) Buffer

A zwitterionic buffer used in a variety of cell culture environments to augment the bicarbonate buffer.

Advantages

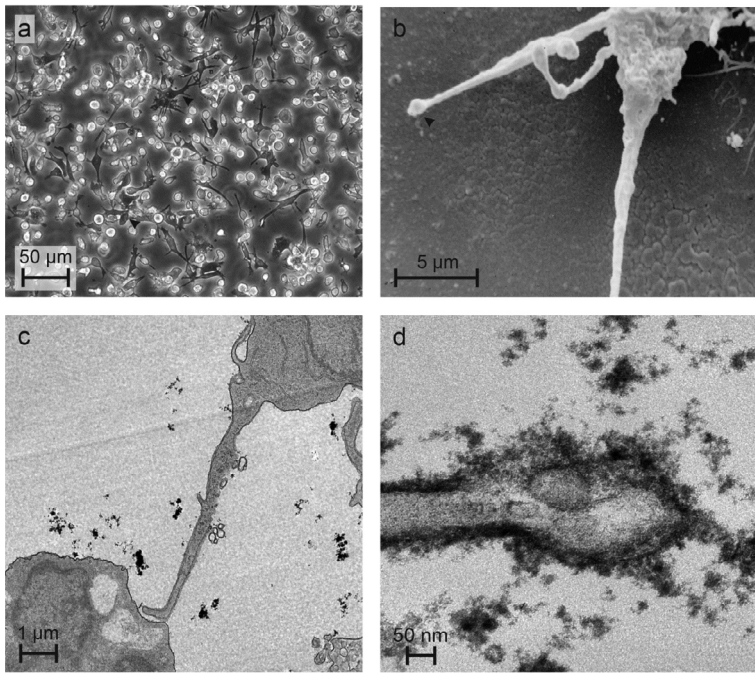
1. Good buffering range between 6.0 – 8.0
2. Limited effect on biochemical reactions and enzymes

Pipes (Good) Buffer

Another common zwitterionic cell culture buffer.

Advantages

1. It minimizes lipid loss in animal and plant samples when buffering glutaraldehyde



Immature dendritic cells growing in conditioned cell culture medium on the surface of fibronectin-coated chambered coverslips. Fig. a shows the appearance of the DC morphology in phase contrast. Fig. b and c show extended cell projections with knob-like structure in SEM (Fig. b) and TEM (Fig. c and at higher magnification in Fig. d). Note the prominent coat of the glycocalyx in Fig. d. In Fig. c paramagnetic particles are visible, which are still present after cell separation but partially detached from the cell surface.

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Quick Comparison Table for Buffers

EMS Cat. No.	Product Description	Unit	Advantages	Disadvantages
Buffers (crystalline)				
21190	Phosphate, Mono basic	500 g	• Non-toxic • No special disposal • Can be custom tailored at a variety of pH ranges • Minimal pH change with temperature • Low cost components/pre-prepared solutions	• Incompatible with calcium ions.
21180	Phosphate, Di basic	500 g		
12300	Cacodylic Acid Sodium Salt	100 g	• Can use with calcium, does not form precipitates • Long shelf life, does not support growths	• Potential carcinogen • Contains arsenic • Controlled disposal required • Expensive
16782	Hepes, Good	100 g	• Buffering range 6.0–8.0 • Limited effect on biochemical reactions and enzymes	
19240	Pipes, Good	100 g	• Minimizes lipid loss in animal and plant samples when buffering glutaraldehyde	
Buffers, Prepared Solutions				
19340-72	Sodium Phosphate, 0.1 M, pH 7.2	1L	• Non-toxic • No special disposal • Can be custom tailored at a variety of pH ranges • Minimal pH change with temp • Low cost components/pre-prepared solutions	• Incompatible with calcium ions.
11582-10	Millonig's, 0.2M	1L		
11600-10	Sorensen's 0.2M, pH 7.2	1L		
11653	Sodium Cacodylate, 0.2 M, pH 7.2	1L	• Can use with calcium, does not form precipitates • Long shelf life, does not support growths	• Potential carcinogen • Contains arsenic • Controlled disposal required • Expensive
11163	PHEM	500 ml	• Very good preservation of cellular ultrastructure • Limited effect on biochemical reactions and enzymes	
11520	S-Collidine	100 ml	• Excellent for buffering osmium tetroxide in 2nd fixation • Very good stability/buffering capacity, max. pH 7.4	• Very strong odor, requires fume hood • Toxic pyrimidine component • Not useful with aldehyde primary fixative
11500	S-Collidine, pH 7.4 Kit	200 ml		
11730-06	Tris 0.2M, pH 8.0	500 ml	• Functional at high pH and temperature ranges used for antigen retrieval • Improves antibody accessibility for immuno histo/cyto chemistry	• Large pH change with temperature, must be adjusted at used temperature
11740	Tris-Maleate, 0.2M, pH 6.4-8.0	500 ml		
11494	Hepes, 0.2M, pH 7.0–8.0	500 ml	• Buffering range 6.0–8.0 • Limited effect on biochemical reactions and enzymes	
11610	Pipes 0.3M	500 ml	• Minimizes lipid loss in animal and plant samples when buffering glutaraldehyde	

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