

## 5.6 Media and Reagents

### A. 0.5% Pepsin Solution

Pepsin	5.0 g
HCl	5.0 mL
Sterile distilled water	to 1000 mL
Store at 4°C.	

### B. 0.5% Trypsin Solutions

#### 0.5% Trypsin Solution

EDTA	0.2 g
NaCl	8.0 g
KCl	0.2 g
KH <sub>2</sub> PO <sub>4</sub>	0.2 g
NaHPO <sub>4</sub>	1.15 g
Trypsin	5.0 g
Sterile distilled water	to 1000 mL
Store at 4°C.	

#### 0.5% Trypsin with Phenol Red

NaCl (sodium chloride)	8.0 g
KCl (potassium chloride)	0.2 g
C <sub>6</sub> H <sub>5</sub> Ha <sub>3</sub> O <sub>7</sub> ·2H <sub>2</sub> O (sodium citrate, dihydrate)	1.0 g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O (sodium phosphate, monohydrate)	0.05 g
NaHCO <sub>3</sub> (sodium bicarbonate)	1.0 g
Glucose	1.0 g
Phenol Red (0.5%)	1.0 mL
Distilled Water	1.0 L
Trypsin	5.0 g

### C. 1N Sodium Hydroxide (NaOH)

NaOH	40.0 g
Distilled water	to 1000 mL

### D. Phosphate Buffered Saline (PBS)

Sodium Chloride (NaCl)	8.0 g
Monopotassium phosphate (KH <sub>2</sub> PO <sub>4</sub> )	0.34 g
Dipotassium phosphate (K <sub>2</sub> HPO <sub>4</sub> )	1.22 g

Distilled water to 1000 mL  
 Filter with 0.22 um filter. Store at room temperature.

### E. Proteinase K

Can be obtained as a stable liquid solution (14 to 22 mg.mL<sup>-1</sup>) from commercial sources. If kept at 4°C, it is stable for > one year.

### F. RNase A

It is a 10 mg.mL<sup>-1</sup> solution and can be obtained from commercial sources [e.g. 5'-3' (Cat. # is 5-888777)] as a 50% glycerol solution that is liquid at -20°C.

### G. DNA Extraction Buffer

The buffer is NaCl 100 mM, Tris-HCl 10 mM, EDTA 25 mM, SDS 1%. The stock solutions are:

NaCl 5M	{50X}
Tris-HCl 1M, pH 7.8	{100X}
EDTA 0.5M, pH 8	{20X}
SDS 20%	{20X}

Stock solutions should be made using ultrapure, nuclease-free water (HPLC grade or equivalent), aliquoted and stored at -20°C until needed. Pre-made stock solutions (molecular biology grade) can be purchased from a commercial supplier, aliquoted and frozen, so the chances of contamination are reduced.

### H. Loading Buffer

Sigma P-7206 Pre-made 6X concentrate, ready to use (store -20°C)

OR	
Bromophenol blue	0.25%
Xylene cyanol	0.25%
Glycerol	30.0%

### I. 0.01 M Phosphate Buffer pH 6.8

Adjust pH of PBS recipe above to 6.8.

### J. 10% Neutral Buffered Formalin

Formalin, concentrated	100 mL
Distilled water	900 mL
Sodium phosphate (monobasic)	4.0 g

Sodium phosphate (dibasic)	6.5 g
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### K. 2.5 mM Magnesium Chloride (MgCl<sub>2</sub>)

It can be obtained from commercial sources and often is supplied with Taq enzyme. Purchase as molecular biology grade.

### L. Leishman Stain

Purchase from commercial source.

### M. Giemsa Stain

Stock solution:

Giemsa powder	1 g
Glycerin	66 mL

Mix and place in oven at 56 to 66°C for 0.5 to 1 hour. Add 66 mL absolute methanol after solution has cooled.

Phosphate buffer, pH 6.0

Sodium phosphate (monobasic)	35 g
Sodium phosphate (dibasic)	4.84 g
Distilled water	to 4 L

Working Giemsa

Stock Giemsa	14 mL
Phosphate buffer	200 mL

Must be made fresh.