

*Reagents in cold room*  
CYTOCHEMICAL IDENTIFICATION OF MONOCYTES

*Enzymes in 5373A freezer*

Cell smears are stored unfixed @ r.t. 2 week maximum

Fixative: pH 6.6 chilled

- 20 mg  $\text{Na}_2\text{HPO}_4$
- 100 mg  $\text{KH}_2\text{PO}_4$
- 30 ml  $\text{H}_2\text{O}$
- 45 ml acetone
- 25 ml formalin (formaldehyde solution)

Procedure:

1. Fix smears for 30 second on ice. Wash 3X distilled  $\text{H}_2\text{O}$  till no formalin smell.  
*air dry at room temp 10 to 30 min. wash well*
2. Prepare reaction mixture
  - A. mix 1.5 ml pararosanilin (4% in 2N HCl) } 1 min mix.
  - 1.5 ml fresh  $\text{NaNO}_2$  (4% in dist.  $\text{H}_2\text{O}$ ) }
  - B. add 44.5 ml Sorenson's phosphate buffer M/15 pH 7.6
  - C. add above buffered reaction mixture to 50 mg  $\alpha$ -naphthyl acetate / 2.5 ml ethylene glycol monoethyl ether.
  - D. check pH 6.1, filter before use.
3. Incubate fixed smears in reaction mixture at room temp for 45 minutes.  
*Whatman # 32, 44, 42, 50, Millipore*
4. Wash with  $\text{H}_2\text{O}$ .
5. Counter-stain with *Methyl Green* hematoxylin 1/2 minutes.
6. Wash with water, dry, mount with permeant or immersion oil.

Enzymic activity seen as dark red granules in cytoplasm of monocytes, histocytes and megakaryocytes.

REAGENTS

1. 2N HCl 16.5 ml of HCl concentrate in 100 ml  $\text{H}_2\text{O}$ .
2. Pararosanilin 4 gm/100 ml 2N HCl.
3.  $\text{NaNO}_2$  4 gm/100 ml of dist.  $\text{H}_2\text{O}$ . Refrigerate (fresh weekly)
4. Sorenson's PBS M/15 (.066 Molar) pH 7.6.
  - 9.08 g  $\text{KH}_2\text{PO}_4$  per liter A
  - 11.88g  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  per liter B
  - or 23.87 g  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  per liter B
5. 20 mg/ml solution of  $\alpha$ -naphthyl acetate in ethylene glycol monoethyl ether.
6. *1.5 gm/100 ml* Naphthol chloroacetate is in 373A white freezer top (insider only)

*Methyl Green Counterstain*

Methyl Green

1 M in 1N Sodium Acetate pH 4.2

Filter before use

Sodium Acetate Buffer, 1N

Stock A = .2 Molar Acetic Acid

Conc acetic acid is 17.4 Molar

to make .2 M add 17.2 ml H<sub>2</sub>O to 2 ml of concentrate

Stock B = .2 M sodium acetate

16.4 gm C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>Na/liter

ADD 36.8 ml A + 21.3 ml B to 58.1 ml of H<sub>2</sub>O for pH 4.2

Conc Acetic Acid mm = 60

$$\% \text{ purity} = 99.9\% = \frac{100 \text{ gm}}{100 \text{ ml}} = \frac{1000 \text{ gm}}{\text{L}} \times 1.06 = 1060 \frac{\text{gm}}{\text{liter}}$$

sp. gr. = 1.06

$$1 \text{ M} = 60 \text{ gm/liter}$$

$$17.4 \text{ M} = 1060 \text{ gm/liter}$$