

เอกสารอ้างอิง

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ภาคผนวก

H. H. H.

HRP - perfusion (Rat)

Rinse - solution (34°C)

1000 ml NSS

0.5 ml (2500U) HEPARIN

Fix - solution (room temp.) 1000 ml

1% PARAFORMALDEHYDE 7M 320 m Osm phosphate buffer (PH 7.4)

1.5% GLUTARALDEHYDE

4% SUCROSE

Postfix - solution (4°C) 500 ml

500 ml Phosphate buffer PH 7.4

50 gm Sucrose

Storing solution

Same as postfix (or add 4% Glutaraldehyde if fixation is bad.)

પરોક્સિડેઝ ટીબી.

Peroxidase TMB

For 125 ml of solution

1. Rinse in distilled water 3x1 minute
2. Pre incubate B to A for 20 minutes
 (A = distilled water 116.6 ml + sodium nitroprusside
 0.125 gm + acetate buffer pH 3.3 6.25 ml)
 (B = 6.25 mg TMB dissolved in 1.8 ml absolute alcohol
 on 40 degree centigrade on heating plate.)
3. Enzymatic reaction for 20 minutes
 Add 2.5 ml of 0.3% H₂O₂
4. Rinse in TMB rinsing buffer 3x1 minute
5. Mount on gelatin slides
6. Dry in room temperature
7. Counterstain with 1% Neutral Red for 1-1.5 minutes.
 (Butanol staining procedure)

Preparation of TMB rinsing Buffer

5 ml of acetate buffer pH 3.3 + 95 ml of distilled water

Acetate buffer pH 3.3

27 gm CH₃COONa.3H₂O + 190 ml 1 M HCl + 810 ml distilled
 water

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Staining with Neutral Red

- | | | |
|----|----------------------|----------|
| 1. | 1% Neutral Red | 1.15 min |
| 2. | Acetate buffer | 2 times |
| 3. | Butanol | 3x1 min |
| 4. | Butanol + Xylene 1:1 | 3 min |
| 5. | Xylene 1 | 2 min |
| | Xylene 2 | 1 min |
| | Xylene 3 | |
| 6. | Mount with permount | |

Acetate buffer

Distilled water 2 litres + Acetate buffer pH 4.8 80 ml

Acetate buffer pH 4.8

500 ml of 0.1 N acetic acid + 750 ml of 0.1 N sodium acetate
filter before using

1% buffer neutral red solution

Add 40 ml of pH 4.8 acetate buffer to each litre of a 1%
aqueous neutral red solution. Filter before using.

ประวัติผู้เขียน

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