หอสมุดกลาง จุฬาลงกรณ์มหาวิทยาลัย

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ภาคผนวก

HRP Technigue

1. Injection (Newly prepared solution 30% HRP)
2. Fix (perfusion) 1% paraformaldehyde
   1.25% glutaraldehude
   0.1 M phoshpate buffer pH 7.4
3. Fix over night in cold room (stir or shake) (not exceeding 1 day)
4. Put in 30% sucrose over night (Store in cold)
5. Cut frozen section 50 µ. collect sections in tray.
6. Pre-incubation in 50 mg. DAB (3,3' Diamino Benzidine tetra HCl) 100 ml Tris HCl buffer pH 7.6 (5 min) (Shake well)
7. Transfer section to new tray. Incubation in 50 mg DAB 100 ml. Tris HCl buffer + 66 µl 30% H_2O_2 (15 min) (Shake well) Section turn brown
8. Transfer section to new tray. Wash 3 time in water, 10 min each.
9. Pick up sections on abluminized slide.
10. Blot dry in air or 37°c
11. For unstain section, clear through xylene and mount
12. For stain section, stain with cresyl violet

Staining Procedure

1. Put slide in xylene (5-10 min)
2. Dry in air (shake in air to blott)
3. Put in 70% alcohol, 5 min and hydrate through water
4. Stain with 0.1% cresyl violet (3-5 min)

5. Wash excess in water.
6. Dehydrate though 100% alcohol
7. Clearing in xylene, and mount

Fixation for HRP method

1. Deeply anesthetize the animal with pentobarbital sodium.
2. Open thorax, cut away pericardium, clearly indentify the left ventricle and right auricle.
3. Insert canula tip into the left ventricle, slowly perfuse with fixative, then cut open the right auricle to drain the blood and fixative
4. Continue the perfusion at appropriate rate of perfusion. (Note the reaction)
5. Remove the brain, (good fixation, demonstrated by hardening of the brain tissues)
6. Store for additional 12 hours in the fixative at 4°c (inrefrigerator)
7. Transfer to 30% sucrose in phosphate buffer and store in the refrigerator till ready for processing.

Fixative (1% paraformaldehyde, 1.25% glutaraldehyde in 0.1 M phosphate buffer)

Preparation of Fixative

Stock paraformaldehyde (2%)

20 gm. paraformaldehyde

950 ml. 0.1 M phosphate buffer

Heat till paraformaldehyde dissolve on a hot plate then filter and add buffer to adjust the volume to 1,000 ml. store in bottle till use.

Before use, add to 1,000 ml. of the stock (2%) solution with 50 ml. of 50 % glutaraldehyde or 100 ml. of 25% glutaraldehyde (Practical grade for light microscope) then dilute with 0.1 M phosphate buffer to the total volume of 2,000 ml.

0.1 M phosphate buffer stock solution

solution A	NaH_2PO_4	27.8 gm.
	H_2O	1,000 ml
solution B	$Na_2HPO_4 7H_2O$	53.65 gm.
or	$Na_2HPO_4 . 12H_2O$	71.7 gm.
	H_2O	1,000 ml.

Working phosphate buffer (0.1 M)

solution A	190 ml.
solution B	810 ml.

Adjust pH to 7.4 and dilute to 2000 ml.

Tris HCl buffer (pH 7.6)

NaCl	7.2 gm.
KCl	0.37 gm.
$MgSO_4 . 7H_2O$	0.25 gm.
Tris	4.48 gm.
Dist. H_2O	1000 ml.

Adjust pH to 7.6 with 0.01 M HCl

ประวัติผู้เขียน

นาง สุพร พลยานันท์ เกิดวันที่ ๘ ตุลาคม พ.ศ. ๒๔๙๕ ที่อำเภอทุ่งสง จังหวัดนครศรีธรรมราช จบการศึกษา วิทยาศาสตร์บัณฑิต สาขาการพยาบาล และประกาศนียบัตรผดุงครรภ์ จากคณะพยาบาลศาสตร์ มหาวิทยาลัยมหิดล เมื่อปี พ.ศ. ๒๕๑๗ ปัจจุบันนี้รับราชการในตำแหน่ง อาจารย์ระดับ ๓ คณะพยาบาลศาสตร์ มหาวิทยาลัยมหิดล.

หอสมุดกลาง