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*Hanker-Yates Reagent (PPD-PC)**

A specific, sensitive, non-carcinogenic substitute for DAB in the demonstration of horseradish peroxidase.

Studies[1,2] have suggested that oxidative coupling reactions of aromatic amines in the presence of phenols might provide a suitable substitute for DAB. These reactions yield deeply-colored synthetic melanin-like compounds which are osmiophilic and sufficiently insoluble to be suitable end products for histochemistry. To utilize such a reaction as a substitute for DAB in the demonstration of exogenous horseradish peroxidase (HRP) tracer, the reaction must be sufficiently rapid to deposit the end-product at the cell or tissue sites of the plant hydroperoxidase alone. This was realized[3] when it was found that the peroxidation of p-phenylenediamine (PPD) was greatly accelerated by the presence of pyrocatechol (PC). The copolymer formed as a result of the oxidative coupling reaction is osmiophilic and bluer than oxidized DAB. It is insoluble and conforms well to biological ultrastructure. The administration of HRP and fixation of tissues for studies in mice, other than those involving axonal transport, are carried out according to the directions of Graham and Karnovsky.[4,5] Instead of DAB medium, cryostat sections or those from an automatic sectioner are incubated in a medium[3] consisting of:

Hanker-Yates Reagent**	7.5 - 15 mg
Tris buffer, 0.1M, pH 7.6	10 ml
Hydrogen peroxide, 1%	0.1 ml

Incubation times for this medium are generally much shorter than those required for adequate staining with DAB. Processing of tissues for demonstration of axonal transport in mouse, rat, cat and monkey may be carried out according to the following procedure developed by Metz.[6]

1. Perfusion solution - 0.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.3, or 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.3.
2. Postfix - 2-10 hours in above fixatives at 4°C.
3. Transfer tissue to 0.1M phosphate buffer plus 30% sucrose at 4°C for 24 hours, or until the tissue sinks in the solution.
4. Cut tissue serially on a freezing microtome at thicknesses ranging from 40 to 80 µm. Collect sections in 0.1M phosphate buffer, pH 7.3, at room temperature.
5. For histochemical reaction, the sections are transferred into:

Hanker-Yates Mixture**	75 mg
0.1M Tris-HCl buffer, pH 7.6	50 ml
1% H_2O_2	0.5 cc

Prepare immediately before using.

6. Incubate sections for 15 minutes at room temperature. Solution will slowly turn brown, but may be reused several times within 1 hour.
7. Transfer sections through 2 rinses of 0.1M phosphate buffer, pH 7.3, for 5 minutes each. Sections can be held for 2 hours before mounting at this point.
8. Mount sections from water-gelatin onto subbed slides and air dry at room temperature. (Sections can be counterstained with 0.1% cresyl violet acetate after an additional 3 day drying period at 40°C.)
9. Dehydrate slides in 100% ethanol, 2 changes 5 minutes each for 40 µm sections and 2 changes 10 minutes each for 80 µm sections.
10. Clear sections in 2 changes of xylene, 6 minutes each. Cover with D.P.X.
    Incubation times for this medium are generally much shorter than those required for adequate staining with DAB.

This reagent is clearly superior to DAB for the demonstration of HRP in cell biology and neuroanatomical studies[3] because erythrocyte staining, which is intense with DAB medium (due to hemoglobin, peroxidase or catalase), is much less prominent. Erythrocyte staining can be completely eliminated by the proper incubation time in a medium with this DAB substitute.

Safety Precautions:

Warning! Causes irritation! Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. Handle under hood with protective gloves and goggles. The full chemical, physical and toxicological properties are not known. Exercise due care. Store at 2-6°C. Keep bottle tightly closed.

** The components of this mixture are non-carcinogenic, but they are skin and respiratory irritants and should be handled with due care. This stable conveniently premixed reagent is ultra purified to insure optium performance.

First Aid:

In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician. Flush skin with water. (Wash clothing before reuse.)

Ordering Information:

Cat. #	Description	Size
08661	Hanker-Yates Reagent	5x1 g amp
00216	Glutaraldehyde, E.M. Grade, snap-open vial, 8% aqueous	30x10 ml amp
00380	Paraformaldehyde, E.M. Grade	1 kg
08389	Xylene, Histology Grade	1 gal

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References:

1. Hanker, J.S., Anderson, W.A., Bloom, F.E., Science, **175**, 991 (1972).
2. Hanker, J.S., and Rabin, A.N., J. Clin. Microbiol., **2**, 463 (1975).
3. Hanker, J.S., Yates, P.E., Metz, C.B., Rustioni, A., Histochem. J., **9**, 782-792 (1977).
4. Graham, R.C., Jr., and Karnovsky, M.J., J. Histochem. Cytochem., **14**, 291 (1966).
5. Graham, R.C., Jr., and Karnovsky, M.J. J. Exp. Med., **124**, 1123 (1966).
6. Metz, C.B., Private communication, Oct. 1977.

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