MYELOPEROXIDASE STAIN

Histochemical Method 100 Tests

INTRODUCTION :

Myeloperoxidase is a lysosomal enzyme localized in the azurophil granules of neutrophils and monocytes. Azurophil granules in granulocytic cells correspond to the relatively large electron dense (primary) granules seen under the electron microscope. The secondary (specific) granules are less electron dense and appear at the myelocyte stage. In the monocytic series the azurophil granules are smaller and are not the first to appear during maturation in these cells. Thus the designation primary for them is not appropriate. The lysosomal granules present in early monocytic cells (monoblasts) are very small and have acid phosphatase but lack peroxidase activity .

Myeloperoxidase can also be demonstrated in the specific granules of eosinophils and basophils. In eosinophils the specific granules are not newly formed but derive from primary granules which are also myeloperoxidase positive. The eosinophil peroxidase has been shown by chemical, cytochemical and immunological methods to be different from that of neutrophils and probably to be under separate genetic control. The enzyme in eosinophils is cyanide-resistant and, in neutrophils, cyanide-sensitive.

Most of the early methods for the demonstration of peroxidase use benzidine and hydrogen peroxide. Probably safer substrates should be considered such as 3,3' –diamino benzidine (DAB) tetrahydrochloride. DAB is the substrate of choice for ultrastructural studies because its oxidized product is electron dense and can be intensified by post-fixation with osmium tetroxide. DAB is also frequently used to visualize the immunoperoxidase reaction. and found reliable in the diagnosis of acute myeloid leukaemia (AML).

REAGENTS :

1.	Fixative : formal-ethanol
2.	Incubation mixture (prepared just before use): a. DAB Content of one Vial b. Buffer 10 ml c. H_2O_2 (substrate) 0.1 ml Add the reagents in this order and mix well after each addition .
3.	Enhancer : Copper sulphate
4.	Counterstain : Methyl green

METHOD :

1. Fix fresh air - dry film of peripheral – blood or bone – marrow films for 1 min. using 20 drops of R1 and then rinse in D. water .
2. Put on the section 10 drops of the incubation mixture (R2) for 1 min. at room temperature .
3. Rinse briefly in D. water
4. Put on the sections 20 drops of the enhancer (R3) for two min.
5. Rinse in D. water .
6. Counterstain in methyl green,put on the section 10 drops of (R4) for 10 min. wash, dehydrate, clear and mount.

RESULT :

Cytoplasm : Dark brown
Nucleus : Green

REFERENCE:

Hanker, J.S., et al . Cancer Research, 39, 1635, (1979)

BIODIAGNOSTIC
DIAGNOSTIC AND RESEARCH REAGENTS

Myeloperoxidase Stain

Histochemical Method
+4 to +8ºC **100 Tests**

CAT. No. **MP 26 11**

FOR RESEARCH USE ONLY

REAGENTS

R1	Fixative	**100**	**ml**
R2a	DAB	**5**	**Vials**
R2b	Buffer	**50**	**ml**
R2c	H_2O_2	**2**	**ml**
R3	Enhancer	**100**	**ml**
R4	Counterstain	**10**	**ml**

CONTACTS

Tele: 02-33385184
Mobil: 0109 – 349 20 77
Fax : 02-33385184 (102)
e.maile : info@bio-diagnostic.com
Website: www.bio-diagnostic.com
Adress: 29 Tahreer St., Dokki, Giza, Egypt