Microscopy

LEUCOGNOST® POX Detection of the peroxidase reaction in leukocytes

Cytochemical reagent kit for the diagnosis of leukemia
This "LEUCOGNOST® POX - Detection of the peroxidase reaction in leukocytes" kit is used for human-medical cell diagnosis and serves the purpose of the hematological and cytological investigation of sample material of human origin.

Air-dry the smears can be stored in the refrigerator for up to 3 days.

The relevant instructions prior to the actual cytochemical reaction.
The smears must be dried in air for at least 30 minutes and be fixed according to diagnostic purposes.

This staining kit is designed for the reaction in the 60-ml Hellendahl cell and contains all reagents required for the detection of the peroxidase reaction in leukocytes.

Sample material
Cyto-centrifuge material, and fresh, native blood or bone-marrow smears should be used as the starting material for all stains. The use of e.g. EDTA as anticoagulant significantly reduces the enzyme reaction. In any case it is not recommended to add any anticoagulant substances.

Reagents
Cat. No. 1.16303.0002
LEUCOGNOST® POX Detection of the peroxidase reaction in leukocytes

Package components:
The staining kit contains
Reagent 1: LEUCOGNOST® POX 4-Chloro-1-naphthol 12 x 75 μmol
Reagent 2: LEUCOGNOST® POX Tris(hydroxymethyl)aminomethane-HCl buffer solution 10 ml
Reagent 3: LEUCOGNOST® POX Hydrogen peroxide solution 5 ml

Also required:
Cat. No. 100974
Ethanol denatured with about 1 % methyl ethyl ketone for analysis ESMERS®

Cat. No. 108562
Aquatex® 50-ml dropping bottle (aqueous mounting agent) for microscopy

Cat. No. 109249
Mayer’s hemalum solution for microscopy 500 ml, 1 l, 2.5 l

Cat. No. 112327
LEUCOGNOST® Fixing Mixture for enzyme cytochemistry 500 ml

Sample preparation
The sampling must be performed by qualified personnel.
Please use thin, air-dried blood or bone-marrow smears that have been stored not longer than three days.
The smears must be dried in air for at least 30 minutes and be fixed according to the relevant instructions prior to the actual cytochemical reaction.

Fix the air-dried blood or bone-marrow smears in LEUCOGNOST® Fixing Mixture

Rinse with running tap water

Air-dry

After fixing the smears can be stored in the refrigerator for up to 3 days.

All samples must be treated using state-of-the-art technology.
All samples must be clearly labeled.
Suitable instruments must be used for taking samples and their preparation.
Follow the manufacturer's instructions for application / use.

Reagent preparation
Preparation of the staining solution
Use only freshly prepared solutions.

For preparation of approx. 60 ml solution mix:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>whole bottle contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>15 ml</td>
</tr>
<tr>
<td>Dissolve reagent 1 in the ethanol and transfer to a 60-ml Hellendahl cell.</td>
<td>45 ml</td>
</tr>
<tr>
<td>Add distilled water with stirring.</td>
<td>10 drops</td>
</tr>
<tr>
<td>Add reagent 2 (tris(hydroxymethyl)aminomethane-HCl buffer solution) with stirring.</td>
<td>2 drops</td>
</tr>
</tbody>
</table>

The prepared staining solution is colorless and remains stable for 3 hours.

Procedure
Staining in the 60-ml Hellendahl cell
The slides must be immersed and moved briefly in the solutions, simple immersion alone yields inadequate staining results.
The slides should be allowed to drip off well after the individual staining steps as a measure to avoid any unnecessary cross-contamination of solutions.
The stated times should be adhered to in order to guarantee an optimal staining result.

Slide with fixed smear
Place in freshly prepared staining solution
Rinse with distilled water
Air-dry
Counter-stain with Mayer's hemalum solution
Rinse with running tap water

Mount, if necessary, with Aquatex® and cover glass.

To enable hematologic specimens to be stored over a period of several months, it is advisable to cover them with an aqueous mounting medium (e.g. Aquatex®) and a cover glass. When left unmounted, the stain remains stable for about 3 days, covered with immersion oil only for a few hours.
The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

Result
As a consequence of the peroxidase reaction, all cells of the neutrophilic and in particular of the eosinophilic maturation sequence from the promyelocytes on are unequivocally peroxidase-positive when black-brown granula are visible.
Also the more mature myeloblasts can contain isolated peroxidase-positive fermet inlets in their cytoplasm; even in the cases of early cell-development stages in which Pappenheim staining does not yet indicate primary granulation. The majority of normal monocytes also show a peroxidase-positive reaction; here, however, compared with the result for the neutrophilic and eosinophilic granulocytes the stain is considerably weaker. Basophilic granulocytes and also all cells of the lymphatic and erythropoietic sequence are peroxidase-negative.

Evaluation
Leukemic blast populations that react partly or completely peroxidase-positive are indicative of acute myeloblastic leukemia (AML). Differential-diagnostics relevant lymphoblasts and lymphoid cells should always be peroxidase-negative, and the Auer rods - which are associated with acute myeloblastic leukemia - will be stained to a conspicuously strong degree. A negative peroxidase reaction does not, however, automatically exclude the presence of acute myeloblastic leukemia.
To facilitate the unequivocal classification of the various forms of acute myeloblastic leukemia (myeloblastic, promyelocytic, and myelomonocytic), an additional esterase reaction must be carried out. If the esterase reaction is less than 50% positive, the exact percentage of peroxidase-positive cells in the respective blast population must be counted. An additional differentiation of the peroxidase/esterase-positive cells by degrees of strength, however, is not necessary here.
Three different reaction types are differentiated in cases of peroxidase-positive leukemia:

POX type 1: up to 5 % POX-positive blasts  
AML without maturation tendency; AUL or ALL not excluded

POX type 2: 5 % to 65 % POX-positive blasts  
AML with maturation tendency or AMMoL

POX type 3: over 65 % POX-positive blasts  
AML with maturation tendency up to APoLoL

AML = acute myeloid leukemia  
AUL = acute undifferentiated leukemia  
ALL = acute lymphoid leukemia  
AMMoL = acute myelomonocytic leukemia  
APoLoL = acute promyelocytic leukemia

**Technical notes**
The microscope used should meet the requirements of a medical diagnostic laboratory. Remove surplus immersion oil before filing.

**Diagnostics**
Diagnoses are to be made only by authorized and trained personnel. Valid nomenclatures must be used. Further tests must be selected and implemented according to recognized methods. Suitable controls should be conducted with each application in order to avoid an incorrect result.

**Storage**
Store the LEUCOGNOST® POX - Detection of the peroxidase reaction in leukocytes kit at +2°C to +8°C.

**Shelf-life**
The LEUCOGNOST® POX - Detection of the peroxidase reaction in leukocytes kit can be used until the stated expiry date.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at +2°C to +8°C.

The bottles must be kept tightly closed at all times. The freshly prepared staining solution is colorless and remains stable for 3 hours.

**Capacity**
The staining kit is sufficient for 12 stainings with up to 16 slides. It is possible to simultaneously stain up to 8 microscope slides or 16 standing back to back in 60-ml Hellendahl cells with extensions (corresponds to one staining preparation).

**Additional instructions**
For professional use only.

In order to avoid errors, the application must be carried out by qualified personnel only. National guidelines for work safety and quality assurance must be followed. Microscopes equipped according to the standard must be used.

**Protection against infection**
Effective measures must be taken to protect against infection in line with laboratory guidelines.

**Instructions for disposal**
The package must be disposed of in accordance with the current disposal guidelines. Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines. Information on disposal can be obtained under the Quick Link "Hints for Disposal of Microscopy Products" at www.microscopy-products.com. Within the EU the currently applicable REGULATION (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 applies.

**Auxiliary reagents**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>100974</td>
<td>Ethanol denatured with about 1 % methyl ethyl ketone</td>
<td>1 l, 2.5 l</td>
</tr>
<tr>
<td>104699</td>
<td>Immersion oil for microscopy</td>
<td>100-ml dropping bottle, 100 ml, 500 ml</td>
</tr>
<tr>
<td>108562</td>
<td>Aquatex® (aqueous mounting agent) for microscopy</td>
<td>50-ml dropping bottle</td>
</tr>
<tr>
<td>109249</td>
<td>Mayer's hemalum solution for microscopy</td>
<td>500 ml, 1 l, 2.5 l</td>
</tr>
<tr>
<td>112327</td>
<td>LEUCOGNOST® Fixing Mixture for enzyme cytochemistry</td>
<td>500 ml</td>
</tr>
</tbody>
</table>

**Hazard classification**
Cat. No. 1.16303.0002

Please observe the hazard classification printed on the label and the information given in the safety data sheet.

The safety data sheet is available on the website and on request.

**Main product components**
Cat. No. 1.16303.0002

<table>
<thead>
<tr>
<th>Reagent</th>
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<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-Chloro-1-naphthol</td>
<td>75 µmol</td>
</tr>
<tr>
<td>2</td>
<td>Tris(hydroxymethyl)aminomethane-HCl buffer</td>
<td>43.4 mmol</td>
</tr>
<tr>
<td>3</td>
<td>Hydrogen peroxide</td>
<td>30 g/l</td>
</tr>
</tbody>
</table>

**Other IVD products**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>101424</td>
<td>May-Grünwald’s eosine-methylene blue solution modified for microscopy</td>
<td>100 ml, 500 ml, 1 l, 2.5 l</td>
</tr>
<tr>
<td>109204</td>
<td>Giemsa’s azur eosiin methylene blue solution for microscopy</td>
<td>100 ml, 500 ml, 1 l, 2.5 l</td>
</tr>
<tr>
<td>111674</td>
<td>Hemaこolor® Rapid staining of blood smear staining set for microscopy</td>
<td>1 set</td>
</tr>
<tr>
<td>116300</td>
<td>LEUCOGNOST® ALPA Detection of the alkaline leucocyte phosphatase activity in leukocytes</td>
<td>12 units</td>
</tr>
<tr>
<td>116301</td>
<td>LEUCOGNOST® EST Detection of the alpha naphthyl acetate esterase reaction in leukocytes</td>
<td>12 units</td>
</tr>
<tr>
<td>116302</td>
<td>LEUCOGNOST® PAS Detection of the periodic acid-Schiff reaction in leukocytes</td>
<td>12 units</td>
</tr>
<tr>
<td>116304</td>
<td>LEUCOGNOST® AP Detection of the acid phosphatase reaction in leukocytes</td>
<td>12 units</td>
</tr>
<tr>
<td>117198</td>
<td>LEUCOGNOST® NASDCL new Detection of naphtho AS-D chloroacetate esterase in granulocytes</td>
<td>12 units</td>
</tr>
</tbody>
</table>

**Literature**