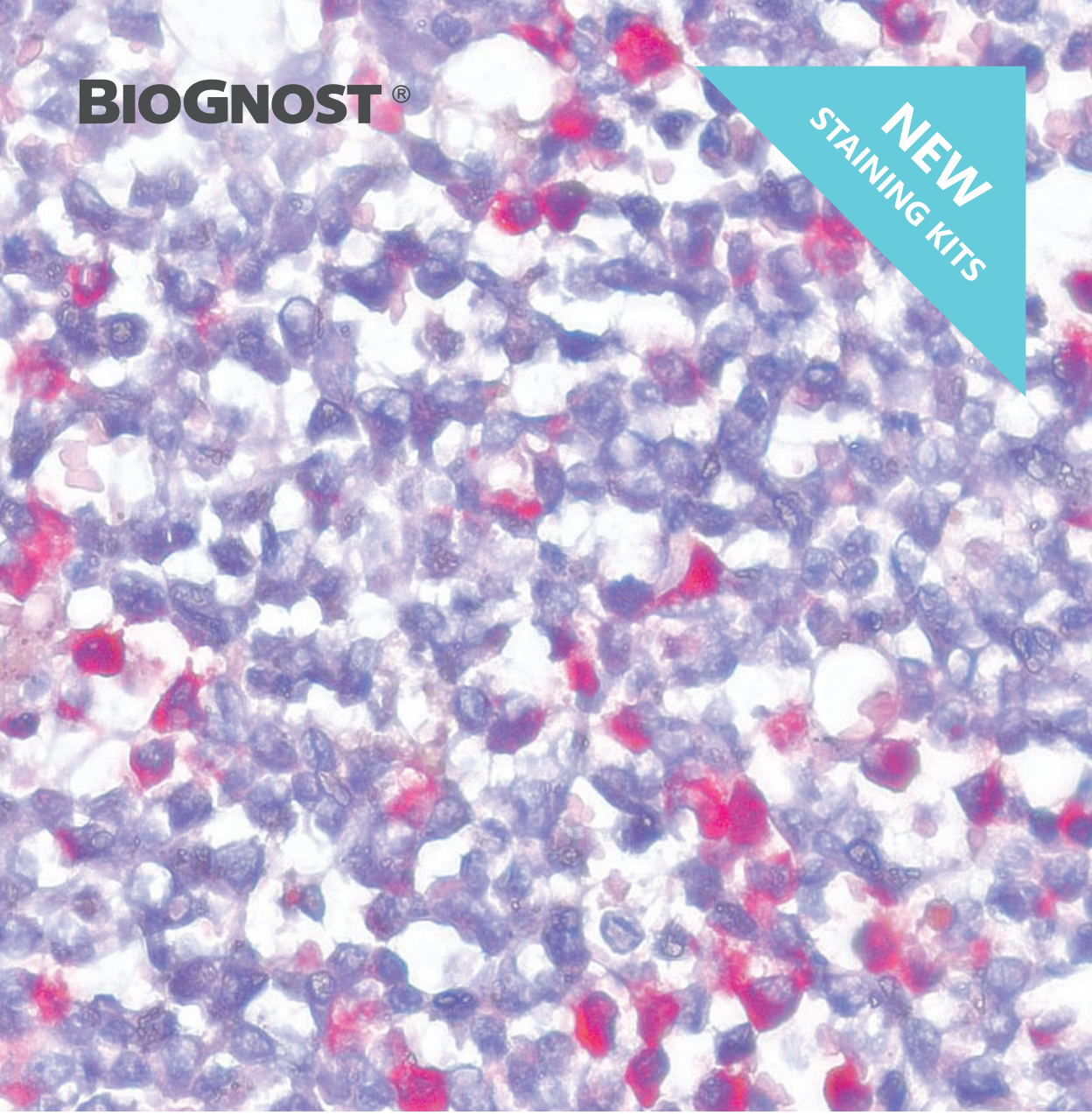
BIOGNOST®

NEW STAINING KITS



# LeukoGnost®

ENZYMOCYTOCHEMICAL STAINING KITS FOR THE DIFFERENTIATION OF LEUKEMIA AND MYELODYSPLASTIC SYNDROMES

# INTRODUCTION

Staining of blood and bone marrow smears is a known method for diagnostics of hematology disorders, used together with modern methods, such as flow cytometry and immunohistochemical techniques. Enzymatic cytochemical staining detects the location and activity of cellular substances and enzyme systems in cytoplasm of leukemia cells, which is important for setting different diagnoses. Besides differentiation of leukemia types, enzyme cytochemical staining techniques can be used in hematological tests for the differentiation of myelodysplastic syndromes (MDS).

Detection of leukemia cells using LeukoGnost kits represents one of the first steps in diagnosing leukemia. Leukemias are neoplastic proliferations of immature cells of the hematopoietic system characterized by abnormal differentiation. Leukemia cells divide rapidly and accumulate in the bone marrow. Replacing normal cells with leukemic cells results in signs and symptoms of the disease. Leukemias can be divided into acute and chronic forms. Acute leukemias originate from primitive hematopoietic stem cells and can be divided into acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). There are 8 types of AML (M0-M7) and 3 types of ALL (L1-L3) that are divided according to morphology, cytochemistry and immunophenotypes. This division enables distinguishing of the lymphoid from the myeloid cell line. There are two types of chronic leukemia: chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL). Chronic leukemia is characterized by replacement of normal bone marrow elements with mature myeloid cells that no longer respond to the mechanisms that control proliferation of normal myeloid cells.

In its portfolio BioGnost offers a complete selection of enzymocytochemical kits for diagnosis and classification of leukemias and MDS, as well as accompanying products (fixative couterstain for nuclei and water-based slide mounting medium). The enzymes whose identification is used in the diagnosis of leukemia are following: phosphatase (alkaline and acidic), esterase (specific and non-specific) and myeloperoxidase. Apart from detecting enzymes, tests that detect the reaction of periodic acid and Schiff's base in cells as well as reagents that cause Berliner Blue reaction are also used for differentiation of leukemias and MDS.

Every LeukoGnost kit is specially formulated as a ready-to-use product eliminating the need to mix or prepare reagents before use. It is up to the user to decide the number of tests to be performed - from one to one hundred tests. There are no working solutions, each test is optimized to use exact amount of reagents needed for the number of tests to be performed.

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# ALP

## Kit for detection of alkaline phosphatase activity in leukocytes (for 100 tests)

Detection of the activity (index) of alkaline leukocyte phosphatase is suitable for the cytochemical differentiation of **chronic myeloid leukaemia (CML)** from myeloid reactions and other myeloproliferative disorders (like myelofibrosis and polycythemia). Index of alkaline leukocyte phosphatase shows different phases of activity of the hematological disease and takes role as a parameter in CML.

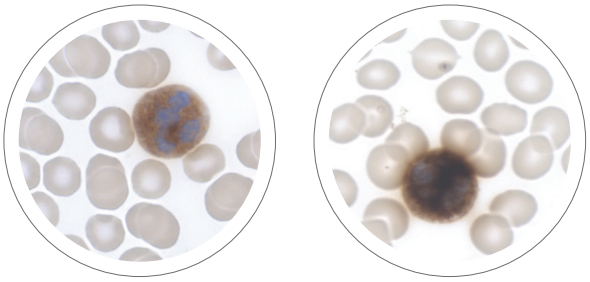
Reagents included with LeukoGnost ALP kit:

| Reagents | | Volume (for 100 tests) |
|---|---|---|
| Reagent 1 | Sodium nitrite, solution | 5 mL |
| Reagent 2 | Variamin Blue B, solution | 3 mL |
| Reagent 3 | ALP buffer | 2x100 mL |
| Reagent 4 | ALP substrate | 2x10 mL |

The staining method is based on the ability of alkaline phosphatase of leukocytes to hydrolyze phosphate esters in alkaline solution. The reaction releases free naphthol that binds to diazonium salts to give a non-soluble brown precipitate at the reaction site. The precipitate is present only in the final mature stages of granulopoiesis. There are five different stages of staining intensity known as Kaplow intensity steps:

| | |
|---|---|
| 0 | no staining |
| 1 | sporadic granules, very weak staining |
| 2 | larger amount of granules that provide weak staining |
| 3 | larger amount of granules that provide moderately strong staining |
| 4 | large amount of granules that provide intensive staining |
| 5 | exceptionally large amount of granules that provide almost homogeneous staining |

Positive reaction in leukocytes with LeukoGnost ALP kit.



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# SPE

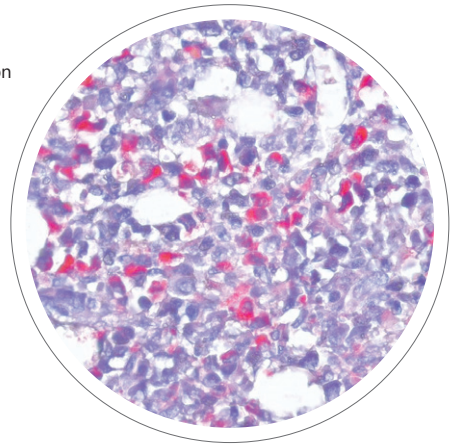
## Kit for detection of specific esterase activity in leukocytes (for 100 tests)

The staining method is based on the ability of the cellular esterase to hydrolyze naphthol AS-D chloroacetate giving positive reaction with cells of the myeloic line in the bone marrow. The reaction releases free naphthol that binds to diazonium salts to give a red precipitate at the reaction site resulting with intense staining of myelocytes, metamyelocytes, stab cells and mast cells. Positive reaction occurs also in myeloblastic leukaemia cells, promyelocytes and Auer bodies, while monocytes show this reaction less frequently. LeukoGnost SPE kit is used to differentiate **acute myelomonocytic leukaemia** (**M4Eo**) and **hypergranular promyelocytic leukaemia** (**M3**) from other leukemias.

Reagents included with LeukoGnost SPE kit:

| Reagents | | Volume (for 100 tests) |
|---|---|---|
| Reagent 1 | Sodium nitrite, solution | 5 mL |
| Reagent 2 | Pararosaniline, solution | 3 mL |
| Reagent 3 | SPE buffer | 2x100 mL |
| Reagent 4 | SPE substrate | 10 mL |

LeukoGnost SPE positive reaction with cells of the myeloic line in the bone marrow.



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# NSE

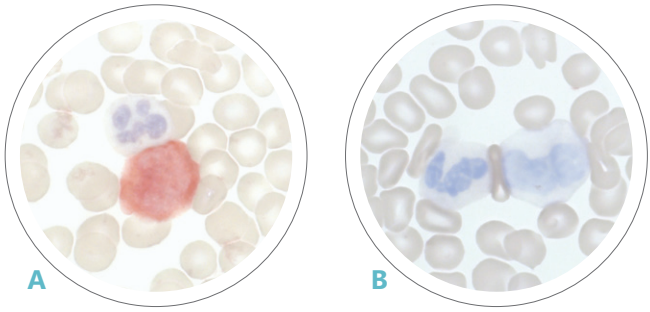
## Kit for detection of non-specific esterase activity in leukocytes (for 100 tests)

Esterase reactions with different substrates enable differentiation between myeloblastic and monoblastic leukemia. The reaction with 1-naphthyl acetate as a substrate is the most suitable for identifying monoblastic types of leukemia (**acute myelomonocytic leukemia and acute monocytic leukemia**).
The staining method is based on the ability of cellular esterase to hydrolyze 1-naphthyl acetate. The reaction releases free naphthol that binds to diazonium salts to give a red-brown precipitate at the reaction site. Monoblasts and monocytes show positive reaction while megakaryocytes and erythroblasts could react weakly. When NSE inhibitor is used, the reaction is completely inhibited while identifying monoblasts. Megakaryocytes and erythroblasts are usually resistant to NSE inhibition with sodium fluoride.

Reagents included with LeukoGnost NSE kit:

| Reagents | | Volume (for 100 tests) |
|---|---|---|
| Reagent 1 | Sodium nitrite, solution | 5 mL |
| Reagent 2 | Pararosaniline, solution | 3 mL |
| Reagent 3 | NSE buffer | 2x100 mL |
| Reagent 4 | NSE substrate | 10 mL |
| Reagent 5 | NSE inhibitor | 3x15 mL |

Blood smears stained with LeukoGnost NSE kit. The samples are stained without (A) or with (B) non-specific esterase inhibitor. Neutrophils and monocytes are shown.



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# SPENSE

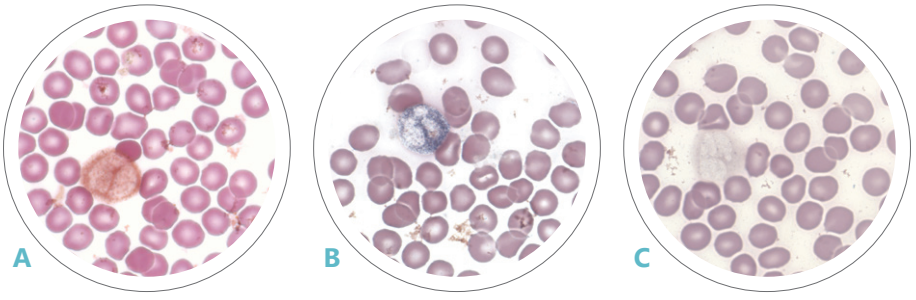
## Kit for simultaneous detection of both, specific and non-specific esterase activity in leukocytes (for 50 tests)

The method is based on the capability of one type of cell esterase to hydrolyze naphthol AS-D chloroacetate and the capability of another type of cell esterase to hydrolyze 1-naphthyl acetate. Both reactions create free naphthols that bind to various diazonium salts to give a non-soluble precipitates at the reaction site. With the LeukoGnost SPENSE kit, neutrophils and myeloblastic leukemia cells turn blue while monocytes turn brownish red. No specific monocyte staining occurs with the use of non-specific esterase inhibitor.

Reagents included with LeukoGnost SPENSE kit:

| Reagents | | Volume (for 50 tests) |
|---|---|---|
| Reagent 1 | Sodium nitrite, solution | 5 mL |
| Reagent 2 | Fast Blue RR/Fast Garnet GBC, solution | 2 mL |
| Reagent 3 | SPE buffer | 100 mL |
| Reagent 4 | SPE substrate | 5 mL |
| Reagent 5 | Fast Garnet GBC, solution | 2 mL |
| Reagent 6 | NSE buffer | 100 mL |
| Reagent 7 | NSE substrate | 5 mL |
| Reagent 8 | NSE inhibitor | 2x10 mL |

Specifically stained monocyte without inhibitor (A), neutrophil (B) and unstained monocyte (C) with non-specific esterase inhibitor are shown.



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# PAS

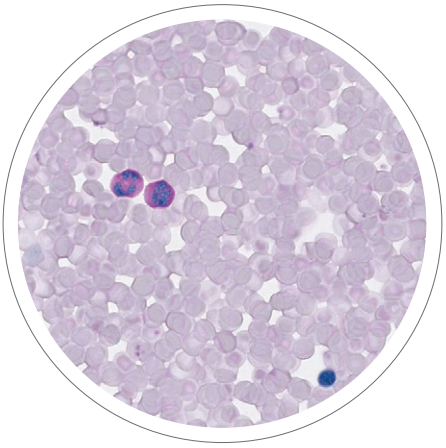
## Kit for detection of periodic acid-Schiff reaction in leukocytes (for 100 tests)

PAS reaction is one of the basic cytochemical staining methods important for diagnosis of **acute lymphatic leukemia** (**ALL**) and **erythroleukemia**. Periodic acid cleaves molecules containing glycol groups to create aldehydes which are affected by the Schiff's reagent in a reaction which stains the cytoplasm magenta. LeukoGnost PAS kit stains neutrophils, monocytes, lymphocytes and basophils in the form of diffuse to fine-grained magenta cytoplasmic stain.

Reagents included with LeukoGnost PAS kit:

| Reagents | | Volume (for 100 tests) |
|---|---|---|
| Reagent 1 | Periodic acid, LeukoGnost | 2x100 mL |
| Reagent 2 | Sodium metabisulfite, LeukoGnost | 2x10 mL |
| Reagent 3 | HCl reagent, LeukoGnost | 2x10 mL |
| Reagent 4 | BioSchiff Forte | 2x100 mL |

LeukoGnost PAS positive reaction with cells of the myeloic cell line in the bone marrow smear.



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# MPO

## Kit for detection of myeloperoxidase activity in leukocytes (for 100 tests)

The myeloperoxidase reaction is used to detect myeloid cell elements where it is possible to obtain a good estimate of the degree of maturity of the maturing granulocytes (cells in the neutrophilic and eosinophilic series) from the intensity of the black color reaction. The staining method is based on the ability of cellular myeloperoxidase to catalyze hydrogen peroxide reduction, creating water and oxygen that oxidizes 4-chloro-1-naphthol, which forms dark blue to black precipitates at the spot of active peroxidase. Leukemic blast population which show positive reaction with peroxidase are evidence of **acute myeloid leukemia (AML)**.

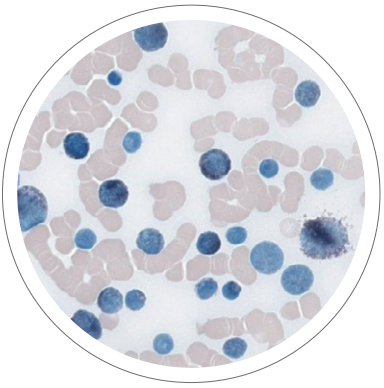
Reagents included with LeukoGnost MPO kit:

| Reagents | | Volume (for 100 tests) |
|---|---|---|
| Reagent 1 | MPO buffer | 2x100 mL |
| Reagent 2 | MPO substrate | 10 mL |
| Reagent 3 | Hydrogen peroxide, solution | 10 mL |

Staining results:

| Cells | Staining |
|---|---|
| neutrophils | intensive black granular cytoplasmic staining |
| monocytes | faint black granular cytoplasmic staining |
| eosinophils | very intensive black granular cytoplasmic staining |
| lymphocytes, basophils | no specific staining |

LeukoGnost MPO positive reaction in the bone marrow smear; acute myeloblastic leukemia.



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# ACP

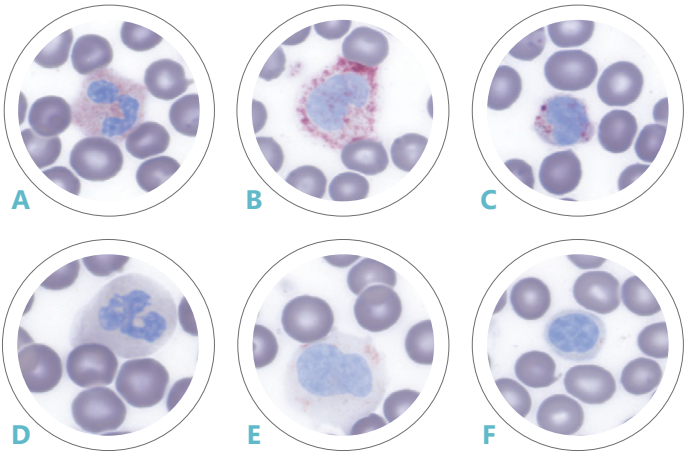
## Kit for detection of acid phosphatase activity in leukocytes (designed for 50 tests)

Acid phosphatase demonstrates specific activity in almost all hematopoietic cells, especially in T-lymphoblastic cells and plasmocytoma cells. The method is based on the ability of the cellular phosphatase to hydrolyze naphthol AS-BI phosphate in acid environment. Solution for staining contains Fast Garnet GBC base and sodium nitrite which form diazonium salts in diazotization reaction. Naphthol AS-BI reacts with diazonium salts and gives a red-brown precipitate at the reaction site of T-lymphoblastic cells. ACP inhibitor (tartrate), inhibits the normal phosphatase activity so that no coloration takes place in the blood and bone marrow cells. LeukoGnost ACP kit is used to diagnose **acute T-lymphoblastic leukemia** in the bone marrow and for diagnosis of **hairy-cell** leukemia.

Reagents included with LeukoGnost ACP kit:

| Reagents | | Volume (for 50 tests) |
|---|---|---|
| Reagent 1 | Sodium nitrite, solution | 5 mL |
| Reagent 2 | Fast Garnet GBC, solution | 2 mL |
| Reagent 3 | ACP buffer | 100 mL |
| Reagent 4 | ACP substrate | 5 mL |
| Reagent 5 | ACP inhibitor | 2x10 mL |

LeukoGnost ACP staining of neutrophils (A,D), monocytes (B,E) and lymphocytes (C,F). Staining conducted without (A,B,C) or with (D,E,F) acid phosphatase inhibitor.



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# PERLS

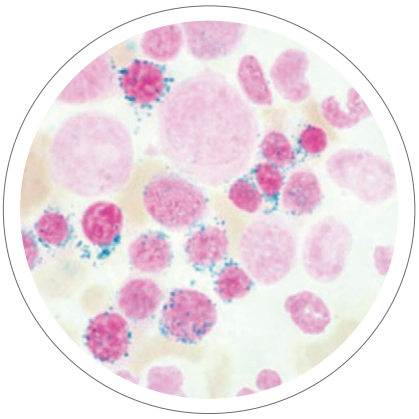
## Kit for detection of free ferric (Fe3+) ions in cells (for 100 tests)

The method of staining is based on the reaction of ferric ion ($Fe^{3+}$), not bound to hemoglobin with potassium hexacyanoferrate (II) in HCl-solution. This reaction is called Berliner blue (Prussian blue) reaction. Product of Berliner blue reaction is bright blue, insoluble precipitate in blood, bone marrow or tissue cells. Myelodysplastic Syndromes (MDS) include different types of **refractory anemia** (**RA**) and **chronic myelomonocytic leukemia** (**CMML**). Berliner blue reaction is positive with ringed sideroblasts, nucleated red cell precursors which on light microscopy have at least five granules of hemosiderin. In the refractory anemia are more than 15 % of all nucleated red blood cells in the bone marrow ringed sideroblasts. Besides in sideroblasts, free ferric ions can be detected in siderocytes, macrophages and endothelial cells.

Reagents included with LeukoGnost PERLS kit:

| Reagents | | Volume (for 100 tests) |
|---|---|---|
| Reagent 1 | Potassium hexacyanoferrate, solution | 30 mL |
| Reagent 2 | HCL reagent, LeukoGnost Perls | 30 mL |
| Reagent 3 | Nuclear Fast Red (Kernechtrot) reagent | 30 mL |

Blue precipitate in granules as a result of Berliner blue reaction.



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# PLUS

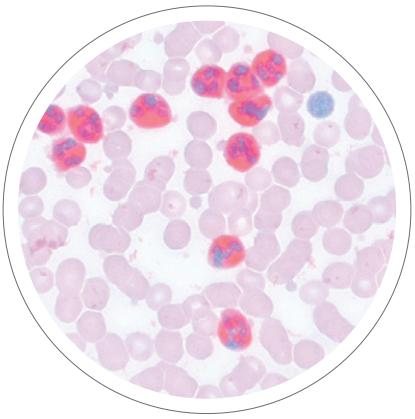
## Additional set of reagents for use with LeukoGnost kits

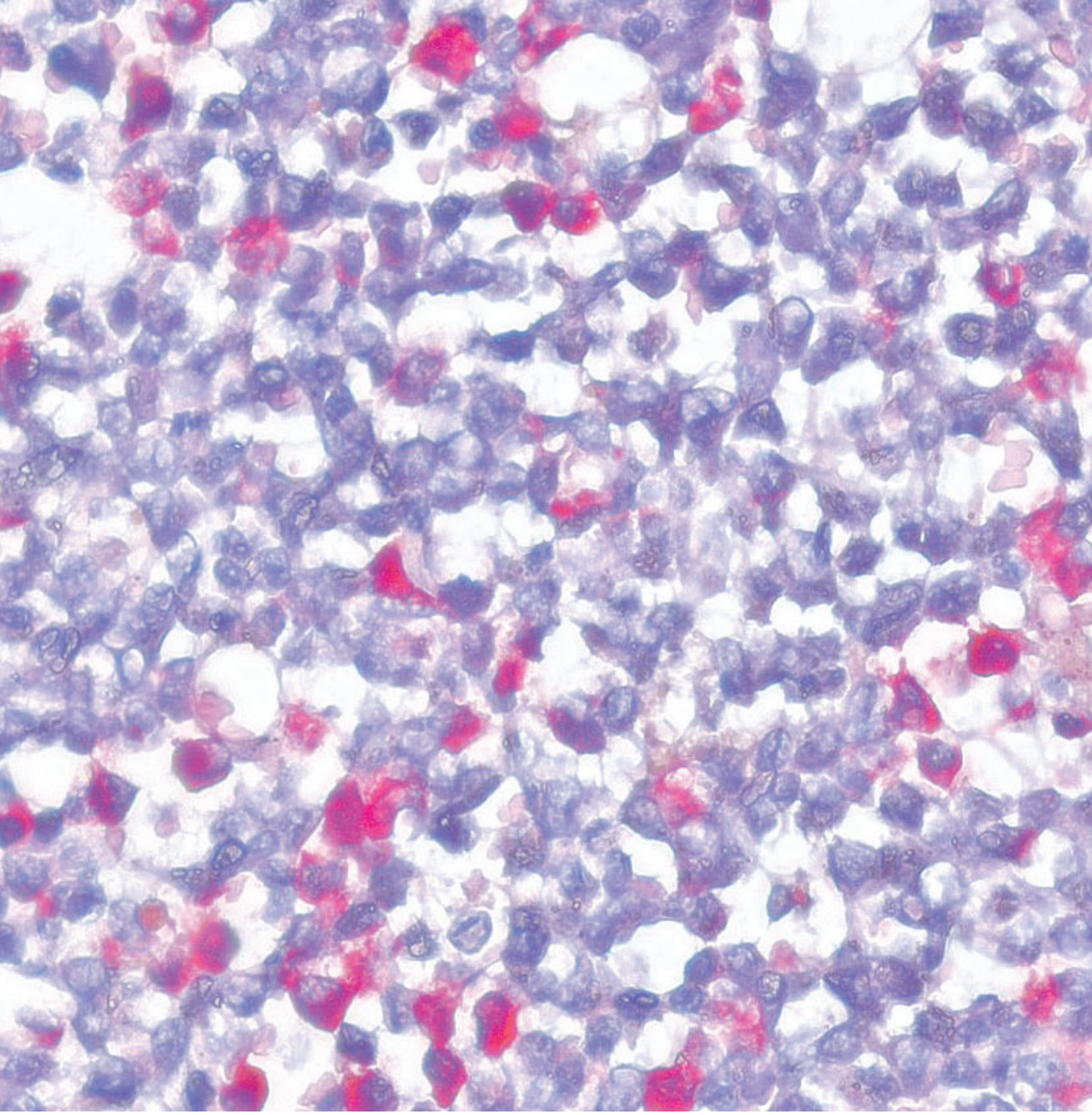
**LeukoGnost fixative** is alcohol free fixative based on acetone and formalin developed specifically for fixing blood and bone marrow used in LeukoGnost kits. The fixative enables optimal enzymatic activity preservation relevant for setting adequate clinical diagnosis. Thin air dried smears from blood or bone marrow must be fresh and the use of EDTA as anticoagulant is not recommended due to its interaction with enzymes.

**LeukoGnost HEM** is a hematoxylin solution used as a nuclei countarstain. This type of hematoxylin is specially designed to complete staining of LeukoGnost kits, not interfering with specific coloration that occurs during staining. Cell nuclei are stained intense dark blue during staining blood and bone marrow smears.

**BioMount Aqua** is a water-based medium for mounting cover slides and permanent storage of stained slides. It provides the expected section transparency by using refractive index similar to refractive index of cover glasses and glass slides; this way the unwanted light refraction is avoided by providing clear and detailed image of the section. It is used for sections that are tested for enzymes and lipids, i.e. for testing samples that should not be dehydrated through alcohol and xylene or xylene substitues.

LeukoGnost SPE positive reaction - smear was fixed with LeukoGnost fixative, stained with LeukoGnost SPE, counterstained with LeukoGnost HEM and mounted with BioMount Aqua.





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