SIGMA-ALDRICH®

SUDAN BLACK B STAINING SYSTEM

(Procedure No. 380)

INTENDED USE

Sigma-Aldrich Sudan Black B Staining System is for use in histochemical demonstration of neutrophil granules in blood or bone marrow films. The Sudan Black B Staining System is for "In Vitro Diagnostic Use."

Several lipids including phospholipids, neutral fats and sterols are stained intensely by Sudan Black B. The reaction of neutrophil granules with the dye was described by Sheehan and Storey in 1947.[1] The leukocyte Sudan Black B staining pattern usually parallels that of myeloperoxidase.[2] Cells committed along lymphoid pathways display negative stain, whereas myeloid and monocytoid forms display characteristic positive reactions. Thus Sudan Black B is considered a useful adjunct in the identification of myelocytic and myelomonocytic leukemias.[2]

Previous methods utilized formaldehyde vapor fixation of blood films.[2] This technique may result in cell loss and staining artifacts. The Sigma-Aldrich procedure utilizes a buffered glutaraldehyde fixative and shortened incubation time that results in excellent staining without cellular loss or distortion.[3]

REAGENTS

SUDAN BLACK B STAINING REAGENT, Catalog No. 3801-200 ml
Sudan Black B, 0.18% (w/v), in 69% ethanol and containing phosphate-buffered phenol.

HEMATOXYLIN SOLUTION, GILL NO. 3, Catalog No. GHS3-100 ml
Certified hematoxylin, 6 g/l, sodium iodate, 0.6 g/l, aluminum sulfate, 52.8 g/l and stabilizer.

GLUTARALDEHYDE SOLUTION, Catalog No. 3802-75 ml
Glutaraldehyde, 4% in borate buffer, pH 7.6.

STORAGE AND STABILITY:

Store Sudan Black B Staining Reagent at room temperature (18–26°C). Reagent label bears expiration date.

Store Hematoxylin Solution, Gill No. 3 at room temperature (18–26°C) protected from light. Reagent label bears expiration date. Filter before use. It is recommended that material be returned to original container after use. Discard if solution turns brown (over oxidized by air) or purple (loss of acidity).

Store Glutaraldehyde Solution in refrigerator (2–8°C). Reagent label bears expiration date. Discard if turbidity develops.

Glutaraldehyde Fixative Solution is stable several months when stored in tightly capped glass bottle in the refrigerator (2–8°C).

PREPARATION:

Prepare Glutaraldehyde Fixative Solution by adding 25 ml of ACS or reagent grade acetone to 75 ml Glutaraldehyde Solution.

PRECAUTIONS:

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet and product labeling for any updated risk, hazard or safety information.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Freshly prepared whole or anticoagulated blood or bone marrow films may be used. Fix as soon as possible.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Acetone, ACS Reagent

NOTES:

It is recommended that blood films prepared from healthy donors be processed along with patient samples as a system-performance check.

The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

PROCEDURE:

1. Cool Glutaraldehyde Fixative Solution in refrigerator (2–8°C).
2. Smears or films of blood or bone marrow preparations are fixed for 1 minute at 2–8°C with gentle agitation followed by thorough rinsing in deionized water.
3. Stain in Sudan Black B Staining Reagent by immersion for 5 minutes with intermittent agitation.
4. Rinse 3 or more times in 70% ethanol until no more dye washes out. This is followed by thorough rinsing in distilled water.
5. Counterstain in Hematoxylin Solution, Gill No. 3, for 5 minutes followed by thorough rinsing in tap water.
6. After air drying, slides may be mounted in DPX Mountant for histology or other suitable permanent mounting medium.

PERFORMANCE CHARACTERISTICS

Neutrophils and their precursors show blue-black intracellular granulation.[2] Monocytes stain less intensely and lymphocytes do not stain with Sudan Black B.[2]

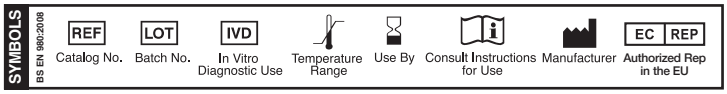
Blood films prepared from normal donors were stained for Sudan Black B according to the described procedure and by the classic Sheehan-Storey method.[1] Neutrophils showed blue-black granulation with Sigma-Aldrich Procedure No. 380, and blue-black granulation with the Sheehan-Storey procedure. In both cases, monocytes stained less intensely and lymphocytes did not show myeloperoxidase activity.

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES

1. Sheehan HL, Storey GW: An improved method of staining leukocyte granules with Sudan Black B. J Pathol Bacteriol 59:336, 1947
2. Davey FR, Nelson DA: Sudan Black B Staining. IN Hematology, 2nd ed. WJ Williams, E Beutler, AJ Erslev, RW Rundles, Editors, McGraw-Hill, New York, 1977, pp 1629–1630
3. Hanker JS, Laszlo J, Moore JO: The light microscopic demonstration of hydroperoxidase-positive phi bodies and rods in leukocytes in acute myeloid leukemia. Histochemistry 58:241, 1978

Procedure No. 380
Previous Revision: 2014-09
Revised: 2016-11



EC REP MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

SIGMA-ALDRICH, INC.
3050 Spruce Street, St. Louis, MO 63103 USA
314-771-5765
Technical Service: 800-325-0250
or e-mail at clintech@sial.com
To Order: 800-325-3010
www.sigma-aldrich.com