

Instructions for Use

SUDAN BLACK IV STAIN

Cat. no. Z83	Sudan Black IV Stain	15ml
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INTENDED USE

Hardy Diagnostics Sudan Black IV Stain is recommended for use in microscopic detection of fecal fat due to malabsorption.

SUMMARY

Sudan Black IV Stain, also known as scarlet red, was introduced by Michaelis in 1901 as a fat stain.⁽⁶⁾ It is a dimethyl derivative of Sudan III, which makes it a deeper and more intense stain, yet it has similar physical properties and is fat soluble.⁽⁶⁾ This stain has been widely used as a screening method, because it is easy to use and correlates well with quantitative methods.⁽⁴⁾

Sudan Black IV Stain is used as a qualitative method to detect the presence of fecal fat. Normally the stool will not contain more than 20 grams of fat daily.⁽⁷⁾ In the case of steatorrhea, fat malabsorption occurs, and greater quantities of fat are detected in the stool. This procedure, when performed carefully and consistently, is a simple method of detecting this condition in the patient.

STAIN FORMULA

Approximate ingredients per liter:

Sudan Black IV, Certified	3.0mg
Ethanol, 95%	740.0ml
Deionized Water	260.0ml

STORAGE AND SHELF LIFE

Upon receipt store at 2-30°C. Products should not be used if there are any signs of contamination, deterioration, or if the expiration date has passed. Do not expose to excessive heat or moisture.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.^(1,5)

For optimum results, fresh, unpreserved fecal material is required. If there is to be a time delay prior to testing, the specimen should be refrigerated immediately after collection. Specimens greater than 48 hours old or specimens that are dried out should be recollected under optimum conditions.^(2,3)

Place a small aliquot of stool suspension on a clean glass slide. Mix two drops of 95% ethanol with the suspension on the slide. Add two drops of Sudan Black IV Stain to the suspension on the slide and mix well. Cover the suspension with a coverslip and examine microscopically for the presence of large orange or red droplets.

INTERPRETATION OF RESULTS

It is recommended that positive controls be run in parallel with patient specimens and that results from this staining procedure be reported only if positive control smears are acceptable.

Neutral fats appear as large orange or red droplets. If 60 or more stained droplets (neutral fats) are seen per 400X (high power) field, then it is a presumptive finding that the patient has steatorrhea.⁽⁴⁾

Fatty acids are present as lightly staining flakes or "needle-like" crystals that do not stain. Soaps will appear as non-staining formless flakes, coarse crystals, or rounded masses.⁽³⁾

LIMITATIONS

Caution must be taken when interpreting the slide, as castor oil and mineral oil may mimic the appearance of neutral fats. Neutral fat globules are generally absent or very rare in normal stool specimens. Therefore, the presence of large quantities of neutral fat may indicate that the patient has ingested mineral oil or castor oil, thus causing a false-positive result.⁽³⁾

Fatty acids may not be visible when they stain as "needle-like" crystals.⁽⁴⁾

Do not count fat present in vegetable cells when reporting results.⁽³⁾

To obtain accurate, repeatable results using the Sudan Black IV staining method, it is very important that only skilled technologists able to maintain very consistent results perform this test.⁽⁷⁾

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as slides, coverslips, microscopes, pipets, applicator sticks, collection bottles, and 95% ethanol, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

Mineral oil or mayonnaise may be used as a positive staining control.⁽³⁾

Water may be used as a negative staining control.

The microscope should be calibrated (within the last 12 months), and the objectives and oculars used for the calibration procedure should be in place on the microscope when objects are measured.^(1,5)

PHYSICAL APPEARANCE

Sudan Black IV Stain should appear dark reddish-orange in color.

REFERENCES

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