

INTENDED USE

Sigma-Aldrich Fetal Hemoglobin reagents are for the acid elution, semi-quantitative determination of fetal hemoglobin in blood smears. Fetal Hemoglobin stain reagents are for "In Vitro Diagnostic Use."

As early as 1864, Korber¹ recognized that the hemoglobin of the fetus was more resistant to alkali denaturation than that of the adult. Advances in techniques for protein isolation and characterization led to the discovery that there are several distinguishing properties that make it possible to differentiate fetal from adult hemoglobin. Among these is the resistance of fetal hemoglobin (hemoglobin F) to acid elution. When blood smears are immersed in acid buffer, for example, adult hemoglobin is eluted from the erythrocytes, whereas fetal hemoglobin is not. If blood smears are treated in this manner and subsequently stained, erythrocytes having hemoglobin F will take up the stain, while those containing only adult hemoglobin appear as "ghosts".

The slide technique for demonstrating fetal hemoglobin in terms of its resistance to acid elution was originally proposed by Kleihauer et al.,² and later modified by Shepard et al.³ The Sigma procedure represents a further improvement in this approach as described by Oski and Naiman.⁴

Fetal hemoglobin estimations are sometimes made to determine possible hemorrhage in the newborn, particularly in cases where there are signs of rectal bleeding. Hemoglobin F assay is also applied to adults as an aid in diagnosing certain types of anemia. For example, from 10–90% fetal hemoglobin is encountered in patients with thalassemia major. Moreover, small increases of fetal blood pigment is usually observed in patients with sickle cell disease.

It is becoming increasingly common in cases of Rh incompatibility to suppress immune reactions to red blood cells entering maternal circulation from the fetus. The amount of specific gamma globulin, containing anti Rh(D) to be administered, is calculated by assessing the magnitude of fetal-maternal hemorrhage.⁵

According to described technique, blood smears, which have been properly dried and fixed, are immersed in a citrate buffer pH 3.3 at 37°C. Adult hemoglobin A (HbA) dissolves out of the cells, whereas fetal hemoglobin (HbF) which is acid resistant, remains intracellular and can be stained for microscopic examination.

REAGENTS

CITRATE PHOSPHATE BUFFER CONCENTRATE, Catalog No. 285-1
Sodium citrate, 0.7 mol/L, and sodium phosphate, 0.6 mol/L.

ACID HEMATOXYLIN SOLUTION, Catalog No. 285-2
Hematoxylin, certified, 1 g/L, aluminum ammonium sulfate, sodium iodate and stabilizers, pH 3.3.

EOSIN B SOLUTION, Catalog No. 285-3
Eosin B, 0.1%, aqueous solution. Sodium azide, 0.1%, added as preservative. Ethanol Fixative, Catalog No. 285-8 (80% v/v ethyl alcohol)

STORAGE AND STABILITY:

Store Citrate Phosphate Buffer Concentrate in refrigerator (2–8°C). Discard if there is evidence of microbial growth.

Store Citrate Phosphate Buffer Solution in refrigerator (2–8°C). Stable for 2 weeks. Use a fresh aliquot each day. Discard if there is evidence of microbial growth.

Store Acid Hematoxylin Solution and Eosin B Solution at room temperature (18–26°C). Solutions may be reused if they are stored in tightly sealed staining jars in subdued light.

Ethanol fixative should be stored at room temperature. Store tightly sealed and as a flammable liquid. Solution may be reused, but should be discarded if fixation is not adequate.

DETERIORATION:

Discard Acid Hematoxylin Solution when the time required for suitable staining exceeds 8 minutes.

PREPARATION:

CITRATE PHOSPHATE BUFFER SOLUTION is prepared by diluting 1 volume of Citrate Phosphate Buffer Concentrate with 9 volumes of water.

Acid Hematoxylin Solution, Eosin B Solution and Ethanol Fixative are ready to use.

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 for any updated risk, hazard or safety information.

US Risk and Safety Statements

Citrate Phosphate Buffer Concentrate is an IRRITANT. Risk of serious damage to eyes. Irritating to respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection.

Acid Hematoxylin Solution is TOXIC. Toxic if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Wear suitable protective clothing and gloves.

Eosin B Solution is HARMFUL. Harmful if swallowed. Sodium azide may react with lead and copper plumbing to form highly explosive compounds.

EDTA Solution is HARMFUL. Harmful by inhalation, in contact with skin and if swallowed. Wear suitable protective clothing.

Ethanol Fixative is FLAMMABLE and an IRRITANT. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition – no smoking. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Target organs: Nerves and liver.

EU Risk and Safety Statements (Caution: Substances not yet fully tested)

Citrate Phosphate Buffer Concentrate is an IRRITANT. Risk of serious damage to eyes. Irritating to respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection.

Acid Hematoxylin Solution is HARMFUL. Harmful if swallowed.

Eosin B Solution is HARMFUL. Harmful if swallowed.

Ethanol Fixative is HIGHLY FLAMMABLE. Highly flammable. Keep container tightly closed. Keep away from sources of ignition – no smoking.

EDTA Solution is HARMFUL. Harmful by inhalation, in contact with skin and if swallowed. Wear suitable protective clothing.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. 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.

Either capillary or venous blood may be used. Capillary blood may be transferred directly to a clean microscope slide. Venous blood should be added to a tube containing EDTA or oxalate. For convenience, use 1–2 drops of 2% EDTA Solution, Catalog No. 285-4, per mL of blood (1 drop = 1 mg). Although blood-EDTA mixtures have been reported to be satisfactory for use up to 2 weeks when refrigerated,³ other studies have concluded that such mixtures should be tested promptly.⁶ Smears should be prepared within 24 hours from blood collected in oxalate.⁷ Using samples from the newborn, it is recommended that the blood be diluted with 0.85% saline, since such specimens have a high content of HbF. Blood smears are not stable and must be tested immediately after preparation.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Microscope
Microscope slides, cover slips
Staining rack/Coplin jars
Water bath, 37°C
Ethanol Fixative, Catalog No. 285-8, 80% v/v ethyl alcohol

NOTES:

For quality control purposes, it is recommended that blood from a normal adult (HbA) and from a newborn or infant (HbF) be included in each series of tests. Barr and Shafer⁸ report that fixed positive control slides from cord blood in EDTA can be preserved up to 1 year at –20°C, in a sealed cardboard box. However, EDTA negative controls do not elute completely after being stored for more than 2 months at –20°C. These investigators suggest preparing positive and negative smears on the same slide; thus, providing clear and rapid contrasts as reference in reading test slides.

Normal Ranges ⁹	
Age	Fetal Hemoglobin (%)
At Birth	50–90
< 2 years	0–4
> 2 years	0–2

Excessive values are observed in:

Aplastic anemia^{3,9}
Erythremic myelosis⁹
Hemoglobin H disease⁹
Hereditary persistence of hemoglobin F^{3,10}
Hereditary spherocytic anemia⁹
Thalassemia major (40–90% fetal hemoglobin)⁹
Thalassemia minor (5–10% fetal hemoglobin)^{3,9}
Sickle cell anemia^{3,9}

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. Citrate Phosphate Buffer Solution should be warmed to 37°C in a Coplin jar or staining dish.
 2. Using clean, labeled microscope slides, make thin blood smears. Prepare CONTROL slides using positive HbF blood (cord-blood) and normal adult blood. Air dry approximately 10 minutes.
 3. Fix slides by immersing in Ethanol Fixative, Catalog No. 285-8, for 5 minutes, rinse thoroughly with tap water and air dry.
 4. Immerse TEST and CONTROL slides in pre-warmed Citrate Phosphate Buffer Solution at 37°C for 5 minutes. Agitate after 1 and 3 minutes of immersion. Degree of agitation may be varied to achieve most desirable results. Rinse thoroughly with distilled water and air dry **completely** to avoid staining artifacts.
 5. Stain the slides for 3 minutes in Acid Hematoxylin Solution, Catalog No. 285-2. Rinse slides with distilled water and shake off excess water.
 6. Counterstain slides for 4 minutes in 0.1% Eosin B Solution, Catalog No. 285-3. Rinse thoroughly with distilled water and air dry.
 7. Place **dry** coverslip on slide and examine using oil immersion (1000X). The absence of HbF is evident by the presence of ghost cells while retained HbF causes cells to appear bright red. Do **not** apply oil directly to slide.
- NOTE: The 400X magnification may be used, but the resulting larger field may be more difficult to count.

PERFORMANCE CHARACTERISTICS

The proportion of erythrocytes containing fetal hemoglobin may be estimated several ways. When studying maternal blood for evidence of HbF-containing cells, Oski and Naiman⁴ recommended the following:

1. Count total number of erythrocytes in 5 fields and determine the average number per field.
2. Then, count the number of deeply stained HbF-containing erythrocytes in about 30 fields and determine the average number per field.
3. Calculate percentage of HbF-containing erythrocytes on the basis of the total number of erythrocytes per field.

Results are reported as the percent HbF present.

Sensitivity studies: According to Oski and Naiman⁴ this method is capable of detecting as little as 0.1 mL of fetal blood in maternal circulation.

Reproducibility studies: Using a series of fresh blood specimens, replicate slides were prepared from each and treated with several different lots of stain on separate occasions.⁵ Microscopic examination revealed essentially identical results with each blood sample.

Correlation studies: Mixtures of cord blood and compatible adult blood were prepared to yield specimens with HbF concentrations ranging from 26–66%.⁶ The blood mixtures were examined by the described technique and assayed chemically by an alkali denaturation method.¹⁰ The percent HbF values showed an average difference of about 7% between methods.

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

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