Pacific Hemostasis®
SickleScreen® Sickling Hemoglobin Screening Kit
or
SickleScreen Control Set

I. Intended Use

Pacific Hemostasis SickleScreen Sickling Hemoglobin Screening Kit and SickleScreen Control Set are intended for use in screening for sickle cell disease and sickle cell trait. SickleScreen Controls can be used with procedures based on differential solubility of reduced hemoglobin, or with enzyme immunoassays specific for Hemoglobin S.

II. Summary and Principles

Sickle cell disease is a chronic hemolytic anemia seen in individuals homozygous for the Hemoglobin S gene (S/S). In these individuals, Hemoglobin S constitutes 70-99% of the total hemoglobin. When Hemoglobin S is reduced to deoxyhemoglobin[1], it forms filamentous tactoids that cause red blood cells of these individuals to "sickle". Repeated vascular occlusion in sickle cell anemia can lead to accumulated damage in a variety of organs, including kidney, heart, lung, and eyes.

Heterozygous (A/S) individuals are carriers of the sickle cell trait and have up to 50% Hemoglobin S. While they are usually asymptomatic, these patients should be identified for genetic counseling purposes. Under conditions of reduced oxygen pressure, such as anesthesia, flight in poorly pressurized airplanes, and severe pneumonia, sickle cell syndrome may occur.

The SickleScreen Kit is a modified Nalbandian[2] procedure based upon differential solubility. Red blood cells are lysed by a surfactant. The released hemoglobin is reduced by sodium hydrosulfite. Reduced Hemoglobin S is insoluble and forms a turbid suspension in concentrated phosphate solutions[3]. Normal Hemoglobin A and most other hemoglobins remain in solution under these conditions. Both sickle cell disease and sickle cell trait can be detected with this procedure.

III. Reagents

For *in vitro* diagnostic use.

A. Reaction Vials (*30 determination kit*): Prefilled with sodium hydrosulfite. Store at room temperature (15-30°C). Do not expose to light for excessive periods. Best stored as supplied in kit. Use uncapped vials within 12 hours.

NOTE: Keep trays protected from light.

Tap the Reaction Vials lightly to break up any clumps and to bring all powder to the bottom of the tube. Do not use Reaction Vials that have clumped, wet-appearing powder. Uncap Reaction Vials and place in Tube Reading Rack. Add approximately 4 mL of Phosphate Buffer to each Reaction Vial. Mix well. Use reconstituted Reaction Vials within 4 hours.

B. Sodium Hydrosulfite Powder Vials: (120 *determination kit*): Store at room temperature (15-30°C). Do not expose to light for excessive periods. Best stored as supplied in kit.

Pour the entire contents of one Sodium hydrosulfite vial into one bottle of Phosphate Buffer. Powder must be dry and free flowing. Mix well and let stand 15 minutes to dissolve. *Store combined Phosphate Buffer/Sodium hydrosulfite tightly capped at **2-8°C***. Use within 28 days of reconstitution. One bottle is sufficient for 30 tests.

Hazard Symbol: Exclamation Mark, Flame
Signal Word: Danger

H302 Harmful if swallowed
H251 Self-Heating; May catch fire

Precautionary Statements - Prevention
Wash face, hands and any exposed skin thoroughly after handling
Do not eat, drink or smoke when using this product
Keep cool. Protect from sunlight
Wear protective gloves/protective clothing/eye protection/face protection
Precautionary Statements - Response Ingestion
IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. Rinse mouth
Precautionary Statements - Storage
Maintain air gap between stacks/pallets
Store bulk masses greater than 1 kg/ 2.22 lbs at temperatures not exceeding .?3 °C/ .?4 °F
Store away from other materials
Precautionary Statements - Disposal
Dispose of contents/container to an approved waste disposal plant
Hazards not otherwise classified (HNOC)
Not applicable
Unknown Toxicity
0% of the mixture consists of ingredient(s) of unknown toxicity
Other information
Harmful to aquatic life with long lasting effects
Interactions with Other Chemicals
No information available.
See Safety Data Sheets for additional information.

C. Phosphate Buffer: A concentrated solution containing surfactant, with 0.02% Sodium azide as a preservative. Store at room temperature (15-30°C).

This chemical is not considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)
Precautionary Statements - Prevention
Obtain special instructions before use
Precautionary Statements - Response
None
Precautionary Statements - Storage
None
Precautionary Statements - Disposal
None
Hazards not otherwise classified (HNOC)
Not applicable
Unknown Toxicity
27.836% of the mixture consists of ingredient(s) of unknown toxicity
Other information
May cause slight eye irritation
Interactions with Other Chemicals
No information available.
See Safety Data Sheets for additional information.

D. Reconstitution Fluid: Deionized water with sodium azide as a preservative. Store at 2-8°C.

***Warning:** Phosphate Buffer and Reconstitution Fluid contain sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Dilute with running water before discarding, and then flush with large volumes of water. These precautions are recommended to avoid deposits in metal piping in which explosive conditions may develop.*

This chemical is not considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)
Precautionary Statements - Prevention
Obtain special instructions before use
Precautionary Statements - Response
None
Precautionary Statements - Storage
None
Precautionary Statements - Disposal
None
Hazards not otherwise classified (HNOC)
Not applicable
Unknown Toxicity
27.836% of the mixture consists of ingredient(s) of unknown toxicity
Other information
May cause slight eye irritation
Interactions with Other Chemicals
No information available.
See Safety Data Sheets for additional information.

E. Positive Control: Lyophilized hemoglobin A/S. Store at 2-8°C. Reconstitute with 0.5 mL Reconstitution Fluid. Let stand undisturbed for 30 minutes then vortex to mix. Reconstituted control is stable for 21 days at 2-8°C.

F. Negative Control: Lyophilized hemoglobin A/A. Store at 2-8°C. Reconstitute with 0.5 mL Reconstitution Fluid. Let stand undisturbed for 30 minutes then vortex to mix. Reconstituted control is stable for 21 days at 2-8°C.

Caution: *Each unit of source material used in the preparation of Positive and Negative Controls has been tested by an FDA licensed method and found non-reactive for HBsAg and negative for antibodies to HIV and HCV. However, no known test method can offer complete assurance that products derived from human blood will not transmit hepatitis, AIDS, or other infectious diseases. This product, like all materials of human origin, should be handled as potentially infectious biological material.*

This chemical is not considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)
Precautionary Statements - Prevention
Obtain special instructions before use
Precautionary Statements - Response
None
Precautionary Statements - Storage
None
Precautionary Statements - Disposal
None
Hazards not otherwise classified (HNOC)
Not applicable
Unknown Toxicity
99.985% of the mixture consists of ingredient(s) of unknown toxicity
Other information
May cause slight eye irritation
Interactions with Other Chemicals
No information available.
See Safety Data Sheets for additional information.

Lack of vacuum (lyophilized controls), unexpected results, or reagent color variations could indicate product deterioration.

IV. Sample Collection

Collect whole blood in EDTA, Heparin, Sodium citrate, or ACD anticoagulant. Samples can be stored at 2-8°C for up to 2 weeks before testing.

V. Procedure

Materials Provided:

Pacific Hemostasis SickleScreen Kit (*30 determinations*):

Phosphate Buffer: 1 x 125 mL vial with dispensing cap

Reaction Vials: 30 vials prefilled with sodium hydrosulfite powder

Pacific Hemostasis SickleScreen Kit (*120 determinations*):

Phosphate Buffer: 4 x 125 mL vials with dispensing caps

Sodium Hydrosulfite Powder: 4 x 5.7 gram vials

Pacific Hemostasis SickleScreen Control Set:

Positive Control: 4 x 0.5 mL vials

Negative Control: 4 x 0.5 mL vials

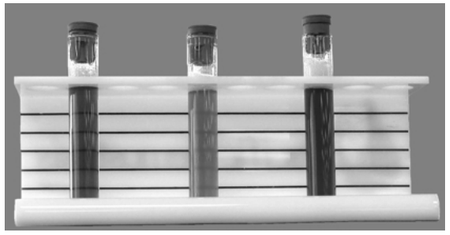
Reconstitution Fluid: 2 x 4 mL vials

Materials Required, But Not Provided:

Tube reading rack
Clear 12 x 75 test tubes and plug stoppers (*120 det. kit*)
50 µL pipet
Controls are not provided with the SickleScreen Kit. They must be ordered separately.

A. Bring all reagents and samples to room temperature.
B. Run a known positive and negative control with each group of samples.
C. Run a positive control with each newly opened tray of tubes.
D. Label one test tube for each patient and control. Use prefilled Reaction Vials for 30 det. kit and 12 x 75 test tubes for 120 det. kit. Place in Tube Reading Rack.
E. In the 30 det/kit, add 4 mL Phosphate Buffer to the prefilled reaction vial and mix well. In the 120 det/kit, add 4 mL Sodium Hydrosulfite to a test tube.
F. Add 50 µL of whole blood or control. Cap and shake vigorously immediately after adding the whole blood or control to each tube.
G. Incubate in Tube Reading Rack at room temperature for 10-20 minutes.
H. **Do not report patient results if the positive control appears negative.**

VI. Results



Negative Weakly Positive Positive

Negative:

If no sickling hemoglobin is present the solution will be clear to slightly cloudy. The lines on the Tube Reading Rack will be easily seen through the tube contents.

Weakly Positive:

The differentiation between weakly positive samples and negative samples soley depends on subjective evaluation. Some will describe a sample giving a slightly cloudy solution with the lines on the Tube Reading Rack visible as very faint lines as positive whereas others describe it as negative. All positive or questionable results should be further evaluated with hemoglobin electrophoresis.

Positive:

If Hemoglobin S or any other sickling hemoglobin is present the solution will be turbid. The lines on the Tube Reading Rack will not be clearly visible when viewed through tube contents. All positive or questionable results should be further evaluated with hemoglobin electrophoresis.

VII. Limitations
A. Severe anemia can cause false negatives. If the total hemoglobin is ≤ 8 g/dL, double the sample volume to 100 µL.
B. Patients with multiple myeloma, cryoglobulinemia, and other dysglobulinemias may give false positives. Wash patient red blood cells in physiologic saline to minimize these problems.
C. Elevated levels of Hemoglobin F can cause false negative results. Do not use this test for infants under 6 months of age.
D. Recent transfusion can cause false positive or false negative results.
E. Some rare hemoglobin variants such as Hemoglobin C Harlem or C Georgetown may give a positive reaction.
F. This test is a screening procedure only. All positive or questionable results should be further evaluated with hemoglobin electrophoresis.
G. Sodium hydrosulfite powder, when exposed to light for excessive periods, may cause false negative reactions.

VIII. Performance Characteristics
Of twenty samples analyzed by hemoglobin electrophoresis, ten were confirmed A/A (≥ 95% Hemoglobin A). The remaining 10 were confirmed A/S (37-46% Hemoglobin S). When tested using the SickleScreen Kit, all A/A samples were correctly reported as negative. All A/S samples were correctly reported as positive. Multiple kit lots were used.

IX. References

1. Lange, R.D., Minnich, V., and Moore, C.V. 1951. *J Lab Clin* Med 37:389.
2. Nalbandian, R.M., Nichols, B.M., Camp Jr., F.R., Lusher, J.M., Conte, N.F., Henry, R.L., and Wolf, P.L. 1971. *Clin Chem* 17:1028.
3. Itano, H.A. 1953. *Arch Biochem Biophys* 47:148.

ORDERING INFORMATION

Cat. No.	Description	Contents
100250	SickleScreen Kit	30 determinations
100258	SickleScreen Kit	120 determinations
503021	Tube Reading Rack	One 10-place rack
100251	SickleScreen Control Set	Positive: 4 x 0.5 mL Negative: 4 x 0.5 mL Reconstitution Fluid: 2 x 4 mL

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Symbols Key

	Manufacturer
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
	Use By
	Temperature Limitation
REF	Catalogue Number
	Consult Instructions for Use
	Exclamation Mark
	Flame

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