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Sickle Cell Assay Kit

(Solubility Assay for Detection of Hemoglobin S)

QBL/PDS/SCD_008

Sickle Cell Assay Kit is a qualitative screening solubility test for the detection of hemoglobin S in blood. For In-Vitro Diagnostic Use only.

ORDER INFORMATION

REF	Pack Size
SKC 50	50 Tests
SKC 100	100 Tests

CLINICAL SIGNIFICANCE

Hemoglobin S (Hb S) differs from the normal Hemoglobin A (Hb A) by a single amino acid mutation at position 6 of the beta chain; wherein glutamic acid is replaced by valine. During low oxygen conditions, the red blood cell morphology may range from mild elongation to irreversible elongated tactoid. This elongated filamentous tactoid formation results in the typical 'sickle' appearance of the red blood cell.

Individuals with sickle cell anemia (homozygous S/S) may have early mortality with vascular occlusions of multiple organ systems, severe hemolytic anemia and hypoxia. Individuals with sickle cell trait (heterozygous NS) are usually asymptomatic. However, under certain conditions of reduced oxygen tension such as hypoxia during anesthesia, flight in poorly pressurized airplanes, severe pneumonia, they can experience a sickle cell crisis.

PRINCIPLE

Sickle Cell Assay Kit for detection of Hemoglobin S is based on the solubility difference between Hb S and Hb A in concentrated phosphate buffer solution. Red blood cells under test are lysed by a powerful hemolytic agent and the released hemoglobin is then reduced by sodium dithionite in a concentrated phosphate buffer. In the presence of Sodium Dithionite, Hb S precipitates causing turbidity of the reaction mixture. Under the same conditions, Hb A, as well as most other hemoglobin's, are soluble. When subjected to a centrifugal force the precipitated hemoglobin (Hb S) forms a red precipitate on top layer leaving the lower solution clear and colorless. The soluble hemoglobin (Hb A) gives a clear red lower solution with a grey precipitate on the top layer and most HbAS which contains both precipitated and soluble hemoglobin gives a red precipitate ring on top layer with a light red to pink color lower solution.

REAGENT

R1- Solubility Buffer
R2-Solubility Powder

STORAGE & STABILITY

Store the reagents at 2°C-30°C. Do not expose to light for excessive periods. The shelf life of the unopened reagents is as per the expiry date mentioned on reagent vial label. After reconstitution the Working reagent (R1) is stable for 1 month at 2°C-8°C and 7 days at 22°C-25°C (do not freeze it). Please refer the working reagent preparation instructions in Reagent Preparation.

REAGENT PREPARATION

Bring the reagents to Room temperature prior to testing.

(a) To prepare working reagent take one Bottle of Solubility buffer (R1) and one vial Solubility powder (R2).

(b) Add sufficient quantity of solubility buffer from R1 Bottle into R2 vial and gently swirl the R2 vial to mix the content well. Transfer the entire content of R2 vial to R1 Bottle. Gently mix the R1 Bottle and allow it to stand for 10 minutes. Label the date of reconstitution on the R1 Bottle and the reagent is now ready to use. After reconstitution store the R1 reagent at 2°C-8°C (Do not freeze or expose to light). Mix the working reagent (R1) thoroughly before use

SAMPLE COLLECTION AND PREPARATION

No special preparation of the patient is necessary prior to specimen collection by approved techniques.

- Collect whole blood in EDTA, Heparin, Sodium Citrate or ACD anticoagulant. Though fresh blood samples are preferable; the sample can be stored at 2°C-8° C for up to 24 hours, in case of delay in testing.
- Prick the finger (preferably ring finger) with the help of lancet. Press the finger till blood oozes out freely. Wipe off the initial blood drop. Using the sample dropper (provided with the kit) collect one drop for the test and immediately add to the labeled Sickle cell tube already filled with 2 mL working reagent.

TEST PROCEDURE

Bring all reagents and samples to room temperature before use.

SCREENING METHOD

1. Use the required number of Sickle cell tubes, as the number of samples to be tested.
2. Label the Sickle cell tubes appropriately and set on the results reading stand.
3. Add 2 mL of the working reagent to each Sickle cell reaction tube with the help of reagent dropper.
4. With the help of a sample dropper, add 1 drop (20 µl) of whole blood sample.
5. Mix well and allow to stand for 15 minutes.
6. To read the test results, place the Sickle cell tubes into the slots of the Result Reading stand provided.
7. Read the turbidity in the Sickle cell tubes by holding the result reading stand against a dim illumination and viewing the black lines printed on the background of the result reading stand, through the solution in the sickle cell Reaction tube.

DIFFERENTIATION METHOD

To differentiate between Sickle cell Trait (Hb AS) and Sickle cell Anemia (Hb SS).

1. If positive results are obtained during the screening method take a fresh Sickle cell tube and repeat the test procedure as in Screening Method with 100 µl of whole blood sample.
2. Mix for 10-15 seconds and allow to stand for 10 minutes.
3. Centrifuge the reaction tube at 1200 rpm for 5 minutes in a laboratory centrifuge.
4. Allow the centrifuge to stop without breaking and carefully remove the test tubes without disturbing the contents.
5. Centrifuge the tube if lower layer is not clear for another 5 minutes.
6. Observe the pattern formed in the Sickle cell tubes.

INTERPRETATION OF RESULTS

Screening Method

1. A turbid solution (black lines on the background of result reading stand are barely visible or cannot be seen) indicates a positive test for sickle cell hemoglobinopathies.
2. A clear solution (black lines on the background of result reading stand are clearly visible) indicates a negative test result.

DIFFERENTIATION METHOD

Type	Lower Layer	Upper Layer
Hb-AA (Normal)	Clear and dark red in colour	Grey precipitate
Hb-AS (Sickle Cell Trait)	Clear and light red to pink in colour	Red precipitate
Hb-SS (Sickle Cell Anemia)	Clear and colourless	Red precipitate

QUALITY CONTROL

Good laboratory practice is recommended for the use of control material along with the test samples to ensure proper performance of the test kit.

PERFORMANCE CHARACTERISTICS

Evaluation of Sickle Cell Assay Kit for detection of Hemoglobin S have yielded good correlation with hemoglobin electrophoresis techniques.

BIBLIOGRAPHY

1. Practical Hematology, Sir John Dacie, Eighth Edition, 1995.
2. Clinical Diagnosis and Management, J.B Henry, 7th Edition, 1998.
3. Diagnostic Hematology by B. F. Rodak, 1995.
4. Clinical Laboratory Diagnostics; Edited by Lothar Thomas, 1st Edition.
5. Standardization in detection of Abnormal Hemoglobin's, R.M. Schmidt *et al.*, JAMA, Vol 225, No.: 10., Sept 3, 1973.

GLOSSARY OF SYMBOL

	Consult Instruction for Use
	Catalog Number
	Store between
	Manufacturer
	Keep away from sunlight

Paramcare Life Sciences Private Limited, G/F-12/13, Evershine-2, Survey No. 307/3/1, Balitha N.H No 48, Vapi, Valsad, Gujarat, 396191.

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