



## **SICKLE-CHECK Screening Test for Haemoglobin-S**

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**Codes: Z2SC101, Z2SC425, Z2SC125.**

The TCS Biosciences Ltd SICKLE-CHECK HbS Test can be performed in two ways.

- a. A screening test to detect sickle haemoglobin (HbS)
- b. A centrifugation test to differentiate the sickle cell trait (HbAS) from sickle cell anaemia (HbSS).

### **SCREENING TEST**

1. Allow the HbS phosphate buffer to reach room temperature.
2. For Z2SC425 and Z2SC125 (4x25 and 1x25 Test Kits) prepare the test solution by dissolving the total contents of one vial of dithionite powder in one bottle of buffer. For Z2SC101 (10x1 Test Kit) prepare the test solution by adding 6ml Hb-S buffer to one sodium dithionite vial. In either case, mix thoroughly. (Any un-used mixture may be stored at 4°C for up to 14 days – the date of reconstitution should be noted on the bottle label).
3. Pipette 2.0ml of the reaction mixture into a 75mm x 12 mm glass test tube and add 20µl of fresh whole blood (EDTA anticoagulated preferable but Heparinised may be used).
4. Mix thoroughly and allow the tube to stand for 5 minutes at room temperature.

**NOTE:** The resulting blood/buffer/dithionite mixture should be a deep purple – red colour (de-oxyhaemoglobin). This colouration should persist. If on the addition of blood to the buffer/dithionite the colour is not as above but is bright red (oxy-haemoglobin) this indicates that the reducing agent has deteriorated. The resultant blood/buffer mixture will then slowly turn to a pale straw colour. If this is the case the test **MUST** be repeated with fresh buffer/dithionite mixture. If in doubt test the effect of mixing blood and buffer without dithionite.

### **THE FAILURE TO RECOGNISE THE DETERIORATION OF THE REDUCING AGENT IS ONE OF THE MOST COMMON ERRORS IN REPORTING FALSE NEGATIVE HbS TESTS**

5. After incubation is complete assess the turbidity of the solution. The presence of sickle haemoglobin (HbS) may be assessed by the inability to read fine newspaper print through the tube or to observe the lines printed on an HbS reading rack through the test solution. A known positive control sample should be assessed with each batch of tests. The reagents have been tested to detect HbS concentrations of about 10% of total haemoglobin content.

False positive results may occur in the presence of abnormal proteins or in hyperlipidaemia, in such conditions the use of washed red cells (50% haematocrit) is advised.

False negatives may be found in cases of severe anaemia, in the new-born infant and during the first months of life. Recent transfusion therapy may also lead to false negative results.

In cases of severe anaemia the blood sample should be centrifuged to sediment the red cells and excess plasma pipetted off to give a haematocrit of 50%. The sample should then be re-mixed and tested as detailed above.

## CENTRIFUGATION TEST

This test is recommended wherever a positive result is found in the screening test (to distinguish heterozygote (HbAS) from homozygote (Hb SS) or in the case of equivocal results in the screening tests. Prepare tube as for screening test and wash into the buffer mixture 0.1 ml of whole blood (haematocrit 40-50%). Mix thoroughly and centrifuge at 1000 rcf for 5 minutes. The centrifuge should NOT be braked. Alternatively the test solution may be filtered through a Whatman No. 1 filter paper. This is desirable if haemolysates are used instead of whole blood. A normal blood and a known positive blood should be tested if available. If a known positive blood sample is not available the test sample itself may be used to allow comparison. In this circumstance the buffer should be diluted 50% with distilled water.

## RESULTS

Absence of HbS A clear or opalescent red solution of reduced haemoglobin showing a greyish protein on the surface.

Haemoglobin AS (sickle trait), SC or SD disease The solution of reduced haemoglobin will be clear and pink. The HbS separates to the surface as a dark red band which is easily distinguishable from the grey protein seen with a normal blood sample.

Haemoglobin SS (sickle cell anaemia) The solution will be clear and straw coloured all the haemoglobin being found as a dark red band at the surface.

When filtration is used in place of centrifugation a red precipitate on the filter paper is indicative of the presence of sickle haemoglobin. The presence of other haemoglobins is indicated by the colour of the filtrate.

**REMEMBER – CHECK ALL POSITIVE OR SUSPECT RESULTS BY CONFIRMING THE PRESENCE OF ABNORMAL HAEMOGLOBIN BANDS ON ELECTROPHORETIC SEPARATION OR BY ANION EXCHANGE CHROMATOGRAPHY.**

## REFERENCES

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