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Sickle-Check Screening Test for Haemoglobin - S

SC101, SC425, SC125, SC510, SC1050* Instructions for use v1.3 09.06.2016

Product identity (name)

SC101, SC425, SC125, SC510, SC1050
Sickle-Check Screening Test for Haemoglobin - S
(synonyms: None)
CAS-No: N/A

Pack Sizes:
SC101 - 1 x 10 test kit = 1 x 60ml SC5000, 10 x 0.1g SC5001
SC125 - 1 x 25 test kit = 1 x 50ml SC5000, 1 x 0.6g SC5001
SC425 - 4 x 25 test kit = 4 x 50ml SC5000, 4 x 0.6g SC5001
SC510 - 5 x 10 test kit = 5 x 20ml SC5000, 5 x 0.4g SC5001
*SC1050 - 10 x 50 test kit = 10 x 100ml SC5002, 10 x 1.2g SC5001.

Product composition

SC5000 & SC5002 - Sickle Buffer
contains sodium azide 0.1% (CAS-No 26628-22-8)

SC5001 - Sodium Dithionite ~100%
(CAS-No 7775-14-6)

For further information see SDS.

Handling and storage

Store at 2 - 8 °C and use before expiry date. The maximum working buffer shelf life of 14 days will be obtained by maintaining the reconstituted solution, tightly stoppered at 2 – 8°C. Repeated warming will shorten the life of the working solution. SC5001 is stable when stored in a cool, dry and well-ventilated place away from direct sunlight.

Intended Use

IVD, for professional use only.

Type(s) of material

Whole blood, or haemolysate samples may be used.

Suggested Procedures [1 - 5]

Principle:

The solubility test for presence of HbS is based on the relative insolubility of HbS when combined with high concentration buffer containing the reducing agent sodium dithionite.
Anticoagulated blood is mixed with the Sickle buffer, the red cells lyse due to the saponin in the buffer and the haemoglobin in the red cells is released.

HbS is insoluble in the high concentration buffer , it forms liquid crystals and gives a cloudy or turbid appearance to the solution. If HbS is not present, the solution will be translucent or transparent.

Turbidity may be assessed by viewing fine newsprint or lines printed on an HbS reading rack through the test solution. If the print cannot be read or the lines cannot be distinguished, the test is deemed positive.

The test can be performed in two ways:
1. A screening test to detect sickle haemoglobin (HbS).
2. A centrifugation test to differentiate the sickle cell trait (AS) from sickle cell anaemia (SS).

Handling and Treatment prior to testing:

It is recommended that a known positive and negative control is assessed with each batch of tests.
Prepare the test solution by dissolving the total contents of one vial of dithionite powder (SC5001) in one bottle of cold buffer (SC5000, SC5002). Mix thoroughly.
For SC101 (1 x 10 test kit) open the vial and discard the grey rubber stopper and then prepare the test solution by adding 6ml HbS buffer (SC5000) to one sodium dithionite vial.

Techniques:

* This kit has been specifically optimised for high volume automated use. Please enquire for further details on procedures.

Method 1. Solubility Screening Test
1. Pipette 2.0ml of the cold dithionite/buffer mixture into a 75 mm x 12 mm glass or plastic** test tube and allow to come to room temperature prior to adding 20µl of fresh whole anticoagulated blood (EDTA is preferable but heparinised may be used).
2. Mix thoroughly and allow the tube to stand for 5 minutes at room temperature.
3. After incubation, assess the turbidity of the solution.

The following test is recommended whenever a positive result is found in the screening test [to distinguish heterozygote (HbAS) from homozygote (HbSS) or in the case of equivocal results in the screening tests].

Method 2. Centrifugation Test
1. Prepare tube as for solubility screening test and wash into the buffer mixture 0.1ml of whole blood (haematocrit 40-50%). Mix thoroughly and centrifuge at RCF 1000 x g 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.

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Method 2. Centrifugation Test Continued.

If a known negative blood sample is not available the test sample itself may be used to allow comparison. In this circumstance the buffer (SC5000 or SC5002) should be diluted 50% with distilled water.

Expected Results [1 - 5]

1. Solubility Screening Test Results

POSITIVE RESULT: The solution is turbid, indicating presence of sickle haemoglobin (HbS).

NEGATIVE RESULT: The solution is transparent.

2. Centrifugation Test 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.

Additional Information

The reagents have been tested to detect HbS concentrations of about 10% of total haemoglobin content.
The blood/buffer/dithionite mixture should be a purple-red colour. This colour should persist. If on the addition of blood to the mixture the colour is bright red, this indicates that the reducing agent has deteriorated, and the solution will slowly turn to a pale straw colour.

In this case, the test MUST be repeated with fresh buffer/dithionite mixture.

The open vial stability of the reconstituted reagent may be adversely affected by repeatedly allowing the reconstituted reagent to come to room temperature. The maximum **working** buffer shelf life will be obtained by maintaining the reconstituted solution, tightly stoppered at 2°C – 8°C. Repeated warming will shorten the life of the working solution.

**Plastic tubes of the same internal dimensions as the specified glass tubes are suitable, provided they are optically clear.

Limitations

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 negative results may be found in cases of severe anaemia, in infants and after recent blood transfusion.
In cases of severe anaemia the blood should be centrifuged and excess plasma removes to give a haematocrit of 50%.

Bibliography

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CE European conformity according to the "IN-VITRO DIAGNOSTIC MEDICAL DEVICES DIRECTIVE 98/79/EC, ANNEX III". Manufactured by TCS Biosciences Ltd.

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