AVONCHEM
LIMITED

SICKLE-TEST

Hb-S solubility screening test kit

INTENDED USE

The Avonchem Sickle-Test can be used to demonstrate the presence of haemoglobin S (Hb-S) in an EDTA-anticoagulated and washed blood samples by the haemoglobin solubility test method.

KIT PRESENTATION

Reagents	100 tests	125 tests
❑ **Reagent A** Buffered saponin	5 × 40 mL	5 × 50 mL
❑ **Reagent B** Sodium dithionite	5 × 0.8 g	5 × 1.0 g
❑ **Viewing card**	1	1

Store the reagents refrigerated (2 to 8°C).

Do not use beyond the expiry date printed on the label.

Required but not supplied:-

Controls

Positive Control (AS)
Washed human red blood cells adjusted to a haemoglobin level of between 12.0 and 15.0 g/L — 0.5 mL

Negative Control (AA)
Washed human red blood cells — 0.5 mL

Store the controls frozen (below -15˚C). Do not use beyond the expiry date printed on the label

The controls contain anti-microbial agents. They are stable for 2 years when stored frozen below -15˚C. They may be thawed and frozen repeatedly without adverse effects. Some users may prefer to prepare aliquots into capillary tubes and remove individual tubes as required

Controls are not included in product. They are available separately

ASSAY PROTOCOL

Preparation

1) Bring the reagents required up to room temperature.

2) Prepare a Working Solution by adding one vial of Reagent B to one vial of Reagent A.

3) Mix well for 5 minutes.

4) Record the date of preparation of the Working Solution on the bottle label. The working solution is stable for up to 4 weeks when kept refrigerated.

Test Method

5) Bring the Working Solution to room temperature.

6) Place 2mL Working Solution into the required number of 75x12 mm test tubes.

7) Add 20 µL (20 mm^3) whole EDTA-anticoagulated blood.

8) Mix well and stand for 3 to 5 minutes.

9) Hold against or 1 cm away from viewing card for best results.

10) Interpret the results for the positive condition. Always use the Positive and Negative Controls for reference: -

Positive	Turbid red solution, partially or completely obscuring the lines on the viewer
Negative	Clear haemolysed solution.

11) Centrifuge the test tubes for 5 minutes at 1000 ×g

12) Interpret the results for homozygosity: -

Homozygous	Yellowish subnatant with a dark red band at the top.
Heterozygous	Red-pink subnatant with a dark red band at the top.
Negative	Slight greyish matter on top of deep red haemolysate.

PROCEDURAL NOTES

The Positive Control and sickle cell positive samples will give a turbid test result. Sickle cell negative samples will give a very slightly hazy result. Since the controls consist of washed cells, the Negative Control will not exhibit the very slight haziness seen with normal fresh blood.

Anaemic Samples. A false negative solubility test result may occur with inadequate quantities of blood taken from anaemic patients. Adjust the haematocrit to approximately 50% by removal of plasma. DO NOT add a double volume of sample.

Neonatal samples. The appearance of Hb-S is genetically delayed and is not present in sufficient quantity until after three months of age. Maximum levels are not reached until about six months of age. Therefore, this test should not be used for testing neonates or children younger than 6 months of age.

False negative results may also occur if the proportion of Hb-S is less than 20%, or following blood transfusion in severe anaemia or if old or outdated reagents are used.

False positive results may be caused by the presence of abnormal plasma proteins or when patients are receiving parental nutrition.

SAFETY PRECAUTIONS

All blood products should be treated as potentially infectious. The blood used in the preparation of the controls was tested and found to be non-reactive for HIV 1, 2 and HCV antibodies and HBsAg. However, no test method can offer complete assurance that products derived from human blood will not transmit infectious agents

REFERENCES

The test is based on the Haemoglobin Solubility Test described by Huntsman *et al*. J Clin Path, 1970;**23:**781-783 and Itano H.A. Arch Biochem Biophys. 1953;**47:**148-59.

Avonchem Ltd
Wellington House
10 Waterloo Street West
Macclesfield
Cheshire
SK11 6PJ
UK

Telephone:
+44 (0) 1625 434300
Fax:
+44 (0) 1625 869777
Email:
james@avonchem.co.uk

Instructions for use edition 01/2010