

**MBP006**

**Hi-Super Speed Sickle Kit  
(Solubility Test for detection of Hemoglobin S)**

**Kit Contents**

Product Code	Reagents/ Materials provided	MBP006
		For 50 Samples
DS0087	Reagent Mix (Pre-weighed for 5 reactions & dispensed in reaction tube)	10 Nos.
ML024	Molecular Biology Grade Water	120 ml

**Other materials provided with the kit**

Empty Reaction Tubes	50 nos.
Test tube stand	1 no.
Dropper	1 no.
Pipette tips	50 no.
Micropipette	1 no.

**Intended Use**

Recommended for identification of normal and sickle cell anemia human blood samples.

**Introduction**

Human hemoglobin is formed from two pairs of globin chains each with a heme group attached. The binding of a heme group into the heme pocket in each chain is vital for the oxygen- carrying capacity of the molecule and stabilizes the whole molecule. Alterations in the structure of hemoglobin are usually brought about by point mutations that affect the coding for amino acids in the globin chains.

In sickle cell anemia, a point mutation (GAG to GTG) in the  $\beta$ - chain at codon position 6 results in the encoding of a valine instead of normal glutamine. The resulting abnormal  $\beta$ - chains combine with normal  $\beta$ - chains to form abnormal hemoglobin S (HbS). HbS is poorly soluble in low oxygen tension situations forming a gel and polymerizing into fibrillary structures or tactoids. This distorts the red blood cells causing them to become rigid and sickled.

**HbA Normal Hemoglobin**

**HbAS Sickle cell trait**

**HbS Sickle cell anemia**

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

### Hi-Super Speed Sickle Kit

This kit is based on the solubility difference between HbS and HbA in Solubility Test Reagent. When red cells are introduced into such a solution, they lyse immediately. The hemoglobin released from the lysed red cells, is reduced by Reagent Mix provided with the kit. This reaction causes precipitation of HbS leading to turbidity of the reaction mixture. However, HbA, as well as other hemoglobins are soluble leading to clarity in the reaction mixture.

This test is simple and stable screening test, however the samples that are tested positive should be confirmed by electrophoresis so as to reduce the chances of False Positives.

### Precautions while handling reagents

1. Reagent for laboratory use only.
2. Do not pipette by mouth.
3. The reagent can be damaged due to microbial contamination or on exposure to extreme temperature.
4. Use reagent of same lot numbers. Do not interchange reagent of different lot numbers.

### Storage

Store the Reagent Mix at room temperature in a cool and dry place. Do not exceed the temperature above 50°C as the powder may absorb moisture and clump. Avoid direct exposure to sunlight. The reagents in the solubility kit have a shelf life of one 1 year (if stored at mentioned conditions).

### Materials needed not provided

1. Centrifuge

### General Preparation Instructions

1. Add adequate amount of Molecular Biology Grade Water (Refer Table 1) to the tube containing pre-weighed Reagent Mix.
2. Vigorously mix the suspension till the solution becomes completely clear.
3. This is the ready to use Solubility Test Reagent.

**NOTE:** The reagent should be freshly prepared before each experiment.

**Table 1:**

No. of Preparations	Volume of clean and clear water to be added in each tube
5	Dispense Molecular Biology Grade Water (ML024) till 10 ml marked on the tube containing pre- weighed Reagent Mix (DS0087) with the help of a dropper (provided with the kit). Make up the volume to 10.5 ml mark after dissolution if required. Allow the bubbles to settle. <b>This will be your Solubility Test Reagent</b>

### Specimen Handling and Collection

Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future). Ensure that the blood sample is at room temperature before beginning the protocol.

## Centrifugation Based Method

### Procedure

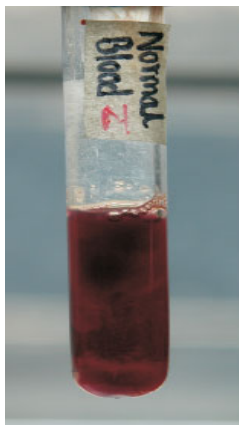
1. Dispense the Solubility Test Reagent with the help of a dropper in an empty reaction tube till the reagent level in the reaction tubes reaches the 2 ml mark indicated on the tube.
2. Gently add a drop of freshly collected whole blood sample to each reaction tube with the help of a Micropipette.

**NOTE:** Anticoagulated blood should be used if the test is not performed on freshly collected blood sample.

3. Gently mix the tubes for 10-15 seconds.
4. Allow the tubes to stand for 10 minutes at room temperature.
5. Centrifuge the reaction tubes at 2,500-3,000 rpm in a tabletop centrifuge for 10 minutes at room temperature (15-25°C).
6. Allow centrifuge to stop without braking and carefully remove the reaction tubes without disturbing the contents.

### INTERPRETATION OF RESULTS

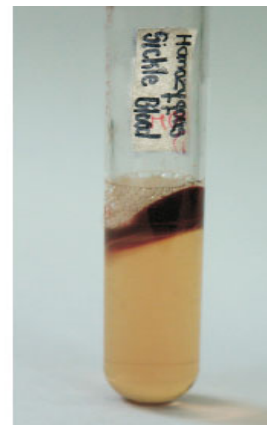
Type of hemoglobin	Lower layer	Upper layer
Hb-A (Normal)	Clear and dark red in color	Grey precipitate
Hb-AS (Sickle Cell Trait)	Clear and light red to pink in color	Red precipitate
Hb-S (Sickle Cell Anemia)	Clear and colourless	Red precipitate



**NORMAL BLOOD**



**HETEROZYGOUS BLOOD**



**HOMOZYGOUS BLOOD**

**NOTE:** This Figure represents single set of experiment. Some slight color variation might be seen.

## Turbidity Based Method

### Procedure

1. Add Molecular Biology Grade Water (ML024) to the tube containing pre-weighed Reagent Mix as indicated in "General Preparation Instructions Table 1" section.

**NOTE:** Mix the tube vigorously for 30 seconds and allow the bubbles to settle. Now the Solubility Test Reagent is ready. **(Refer to General Preparation Instructions)**

2. Place Empty Reaction Tubes in the Test Tube Stand (provided) and dispense the Solubility Test Reagent with the help of a dropper in 5 empty reaction tubes till the reagent level in the reaction tubes reaches the 2 ml mark indicated on the tube.
3. Gently add 1 drop of freshly collected whole blood sample to each reaction tube with the help of a Micropipette.

**NOTE:** Anticoagulated blood should be used if the test is not performed on freshly collected blood sample.

4. Gently mix the tubes for 10-15 seconds.
5. Allow the tubes to stand for 10 minutes at room temperature.
6. Place the tube in the Test Tube Stand provided with the kit and read for turbidity.

**Interpretation of Results: Place the test tubes in the test tube stand and observe the results in a well-lit area.**

- I. Negative- If solution is clear and black lines visible.
- II. Positive- If Solution is turbid and black lines not visible.

Sr. No	Turbidity	Clarity	Visibility of black lines through the tubes	Interpretation
I	No	Yes	Yes	Normal
II	Yes	No	No	Sickle cell



**Remarks**

1. All positive results should be confirmed by running agarose gel electrophoresis (MBP001 or MBP008).
2. The results of the test should be correlated with clinical findings to arrive at the final diagnosis.

**Warning**

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

**Precautions**

Read the procedure carefully before starting the experiment.

**Performance and Evaluation**

Each lot of HiMedia's Hi- Super Speed Sickle Kit is tested against predetermined specifications to ensure consistent product quality.

**Quality Control**

Type of hemoglobin	Lower layer	Upper layer
Hb-A (Normal)	Clear and dark red in color	Grey precipitate
Hb-AS (Sickle Cell Trait)	Clear and light red to pink in color	Red precipitate

Type of Sample	Turbidity observed	Clarity
Normal blood sample	No	Yes
Sickle blood sample	Yes	No

- I. **Negative- If solution is clear and black lines visible.**
- II. **Positive- If Solution is turbid and black lines not visible.**

**NOTE: Each test should be performed with known positive and negative control**

**Limitations of the test**

1. Conditions like severe anemia (hemoglobin level less than 7 gm/dL) can result in false negatives.
2. Fetal hemoglobin more than 25% can result in false negative results.

**References**

1. A rapid whole blood solubility test to differentiate the sickle-cell trait from sickle-cell anemia R. G. HUNTSMAN, G.P.T.BARCLAY , D.M.CANNING , AND G.I.YAWSON.
2. Practical Hematology, Sir John Dacie, Ninth edition, 2001.
3. Clinical Diagnosis and Management, J.B Henry , 20<sup>th</sup> Edition , 2001.

**Safety Information**

Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.












**Disposal**

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.


**Technical Assistance**

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, send an email to [mb@himedialabs.com](mailto:mb@himedialabs.com).

## Symbols

	Manufacturer		Do not use if package is damaged
	Authorized representative in the European Community		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		In vitro diagnostic medical device
	Batch code		CE marking of conformity
	Catalogue number		

### Authorized representative (AR) Address :

	<b>AR Experts B.V.</b> Boeingavenue 209, 1119 PD, Schiphol-Rijk, The Netherlands
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Identification No.: PIMBP006

Rev.No.:09

Date of Issue: 2026-01

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