# MBP006

## Hi-Super Speed Sickle Kit

**(Solubility Test for detection of Hemoglobin S)**

### Kit Contents

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Reagents / Materials provided</th>
<th>MBP006 For 50 Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS0087</td>
<td>Reagent Mix (Pre-weighed for 5 reactions &amp; dispensed in reaction tube)</td>
<td>10 Nos.</td>
</tr>
<tr>
<td>PW1170</td>
<td>Test tube stand</td>
<td>1 No.</td>
</tr>
<tr>
<td>PW1206</td>
<td>Droppers</td>
<td>50 Nos.</td>
</tr>
<tr>
<td>ML024</td>
<td>Molecular Biology Grade Water</td>
<td>150 ml</td>
</tr>
</tbody>
</table>

### Following materials will be provided with the Kit:

- Empty Reaction Tubes - 50 nos.
- Lancets - 50 nos.
- Alcohol swabs - 50 nos.
- EDTA tubes - 5 nos.
- 25µl Micropipette - 1 no.
- 10µl tips - 50 nos.
- Droppers 3ml - 2 nos.
- 2 ml Syringes - 5 nos.
- Labels - 60 nos.

### 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 β-chain at codon position 6 results in the encoding of a valine instead of normal glutamine. The resulting abnormal β-chains combine with normal β-chains to form abnormal hemoglobin S (HbS). HbS is poorly soluble in low oxygen tension situations forming a gel and polymerizing into fibrillar 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 45°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 13 months (if stored at mentioned conditions).

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:**

<table>
<thead>
<tr>
<th>No. of Preparations</th>
<th>Volume of clean and clear water to be added in each tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>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</td>
</tr>
</tbody>
</table>

Sample Collection

Clean the area using Alcohol swab and allow to dry completely before pricking.

Follow either of the following two procedures for sample collection:

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

2) Prick the finger (preferably ring finger) with the help of lancet. Press the finger till blood oozes out freely. Discard the initial 1-2 blood drops. Using the dropper, collect one blood drop for the test and immediately add to the reaction mixture (see “Procedure” below)
Procedure

1. Add Molecular Biology Grade Water (ML024) to the tube containing pre-weighed Reagent Mix as indicated in “General Preparation Instructions” 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 (PW1170) (provided) and dispense the Solubility Test Reagent with the help of a dropper in 5 reaction tubes till the reagent level in the reaction tubes reaches the uppermost black line marked on the test tube stand provided with the kit.

3. Gently add 1 drop of freshly collected whole blood sample to each reaction tube with the help of a dropper (PW1206) (provided with the kit).

   **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 (PW1170) 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.

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Turbidity</th>
<th>Clarity</th>
<th>Visibility of black lines through the tubes</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Normal</td>
</tr>
<tr>
<td>II</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Sickle cell</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
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 Invitro Diagnostic Use (IVD). Not for Medicinal Use.

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

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Turbidity observed</th>
<th>Clarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal blood sample</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Sickle blood sample</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

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

Limitations of the test

1. Conditions like severe anemia (hemoglobin level less than 7 gm/dL) can result in false negatives.
2. Foetal 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 anaemia R. G. HUNTSMAN , G.P.T.BARCLAY , D.M.CANNING , AND G.I.YAWSON.

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 proper cleaning of equipment and floors with plenty of water. Offer surplus and non-recyclable solutions to a licenced disposal company.

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.