Enzyme Immunoassay

Zeta Globin

Cat # 1025Z

For in vitro Research Use Only

NAME AND INTENDED USE
The Diagnostic Automation ZETA GLOBIN ASSAY (ZAM) is a solid phase enzyme linked immunosorbent assay. This test provides rapid screening for the determination of elevated Zeta globin levels in whole blood to aid in the detection α-thalassemia-1 carrier resulting deletion. (For Professional Use Only)

SUMMARY AND EXPLANATION OF TEST
Alpha thalassemia is by far the most prevalent genetic disorder of humans\(^1\). Alpha thalassemia is a hereditary disorder in which alpha globin chains synthesis is either decreased or absent. Patients with alpha thal synthesize abnormally low amounts of alpha globin chain and hence synthesize abnormally low amounts of hemoglobin. DNA mutations that are inherited cause alpha thalassemia. Four alpha globin genes are involved in alpha chain production, two on each chromosome. Any of one, two, three or four genes can be missing. Although 40+ mutations have been discovered to cause alpha thal mutations is the one that puts patients at greatest risk. The most common alpha-thalassemia mutation in Southeast Asia or Southern Chinese populations is the mutation double alpha-globin deletion\(^2\).

Carriers of double alpha-globin deletions are “at risk” to bear: 1.) a child afflicted with HbH diseases (three gene deletion) or 2.) a fetus afflicted with hydrops fetalis syndrome (four gene deletion). Furthermore, pregnancies involving hydrops fetalis syndrome are associated with an increased risk of maternal complications such as hydramnios, preeclampsia, antepartum or post partum hemorrhage, and difficult vaginal delivery\(^3\).

The deletion spares the embryonic zeta globin genes and carries traces of zeta-peptide to persist throughout life\(^2\). Hence in deletion carriers, low levels of zeta globin chains circulate in erythrocytes. In
normal adults, no zeta globin chains circulate in erythrocytes. In almost all infants older than 3 most of age, zeta globin chains are not detected\(^4\). Zeta globin is the embryonic form of the alpha chain of hemoglobin\(^5\). Zeta globin chains that can be detected by antibodies provide rapid, simple, and reliable screening for the double alpha-globin deletion\(^6\).

**PRINCIPLE OF THE ASSAY**

The DAI Globin Assay is a solid phase enzyme linked immunosorbent system employing plastic wells coated with peptide antibodies. Incubation of blood sample in the coated wells results in the binding of peptide to the immobilized antibodies. Subsequent addition of the enzyme conjugate, comprised of horseradish peroxidase, results in the formation of peroxidase, antibody-antigen complex on the solid phase. Unbound enzyme conjugate is washed from the wells and a substrate and chromogen solution are added. The color developed indicates the presence z peptide in the sample, a solid phase enzyme linked immunosorbent assay.

**WARNINGS AND PRECAUTIONS**

1. DAI Globin Assay is a qualitative test. It is designed for in vitro diagnostic use only
2. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
3. Warning potential bio-hazardous material: The Negative and Positive controls is human whole blood. The whole blood found negative for HIV, HCV and hepatitis B antibodies when tested with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HBsAg, HIV, HCV, or other infectious agents are absent, these reagents should be handled at Biosafety level 2, recommended for any potentially infectious human serum or blood specimen in the Center for Disease Control/ National institutes of Health Manual, “Biosafety in Microbiological and Biomedical Laboratories” 1984.

**STORAGE AND STABILITY**

1. Store the kit at 2-8°C in a refrigerator. Keep micro-wells sealed in dry bag with desiccants.
2. The reagents are stable until expiration of the kit.
3. Solution A and Solution B should be colorless; if the solution turns blue, it must be replaced. Do not expose test reagents to strong light during storage or usage.

**SAFETY INSTRUCTIONS**

1. Negative and Positive Control are form human whole blood and found to be negative for HBsAg, HIV and HCV. However, for safety, it must be treated as infectious materials.
2. Do not smoke or eat in areas where specimens or reagent kits are handled.
3. Do not mouth pipette. Wear PVC gloves when handling reagent kits or specimens, and wash hands thoroughly afterwards.
4. Infectious specimens and non-acid-containing spills should be wiped up thoroughly with 5% sodium hypochlorite solution.
5. All waste material should be properly disinfected before disposal. Both liquid and solid waste can be autoclaved for at least one hour at 121.5°C. Solid waste can also be incinerated. Non-acidic liquid waste requires neutralization before similar treatment and should stand for 30 minutes to obtain effective disinfection.
6. Avoid contact of hydrochloric acid with skin and mucous membranes.
MATERIALS PROVIDED
1. Microwell strips (96 wells): peptide antibody coated wells. (12x8)
2. Sample Diluent (20 mL): lysing reagent for Red blood cells.
3. Washing concentrate (50 mL): 1 bottle, prepare washing buffer by adding distilled water to one liter.
4. Enzyme conjugate (11 mL): Anti- Antibodies conjugate with horseradish peroxidase.
5. Negative Control (0.3 mL) containing no blood.
6. Positive Control (0.3 mL) containing blood.
7. Solution A (11 mL): Buffer solution containing hydrogen peroxide.
8. Solution B (11 mL): Tetramethylbenzidine solution.
9. Stop Solution: 2 N HCl.
10. Well holder: For securing individual wells.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Micro-well reader at 450 nm.
2. Pipetor with tips for 25 uL & 100 uL
3. Water bath or incubator with temperature control (37°C)

SAMPLE COLLECTION AND HANDLING
Collect blood aseptically by venipuncture, in lavender (EDTA), gray or blue top tube. The whole blood can be assayed immediately or they can be stored at 2-8°C for up I week or frozen at -20°C for up to 30 days prior to assay. Sample may be also be frozen for up to 3 years at -70°C. Hemolyzed blood sample is ideal for the assay.

PREPARATION FOR ASSAY
1. Before beginning the test, bring all samples and reagents to room temperature and mix each gently.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruption to get the most reliable and consistent results
3. Use new disposable tips for each sample.

ASSAY PROCEDURE
1. Secure the appropriate number of test glass tube (12x75 mm). Record the identification of each tube to maintain specimen identification.
2. Dispense 200 uL of sample diluent into each test tube. Add 500 uL of patient sample, reference standard (or controls) to each respective tube.
3. Vortex each tube vigorously for 20 seconds to make sure that red blood cells lyse completely. Set these aside until step 5.
4. Secure the desired number of coated wells in holder. Record the identification of each well
5. Transfer 100 uL of the above treated sample to maintain specimen identification of each respective well in duplicate.
6. Incubate at 37°C for 30 minutes. Invert wells to decant incubation mixture. Tap wells to ensure thorough removal of incubation mixture.
7. Rinse the wells 5 times with wash buffer.
8. Dispense 100 uL of enzyme conjugate into each well and mix for 5 seconds and incubate in 37°C for 30 minutes.
9. Remove mixture and rinse the wells 5 times with wash buffer gently. (Be sure to wash the wells thoroughly and completely dry the wells. Improper wash may cause false positive results).
10. Dispense 100 uL of Solution A and 100 uL of Solution B into each well. Mix it for 5 seconds and incubate in the dark for 15 minutes at room temperature (22-26°C)
11. Stop reaction by adding 50 uL of 2 N HCl solution to each well and read at 450 nm with micro-well reader against Blank well (only Solution A and Solution B).

RESULTS:
1. Negative Control: optical density should be below 0.2 A.U. Normal patient will have zero chain.
2. Positive Control: The optical density should be no less than 0.5A.U that should contain chain in the samples.

QUALITY CONTROL
Results of an assay run are valid if the following criteria are met: The mean absorbance of Negative Control should be less than 0.2. The absorbance of the Positive Control should be more than 0.5. Each laboratory should utilize internal controls several levels to monitor assay performance. The controls should be treated as unknown. Results obtained should be in agreement with the assigned values.

EXPECTED VALUES AND INTERPRETATION
Compare the color of the patient samples well to the color of the positive and the negative reference wells.
Negative: Samples that developed no color or less intensity than 0.2 A.U. are considered negative in DAI Assay.
Positive: Samples that developed the color equal to or stronger than 0.3 A.U. are considered Positive.
Borderline: Samples with O.D. between 0.2 to 0.3 are considered borderline. Repeat assay. If O.D. is below 0.25 report as negative. If O.D. is greater than 0.25, run PCR to confirm the result.

APPLICATIONS & LIMITATIONS
DAI Zeta Globin Assay detects α–thal-1 carriers resulting from the deletion. It also detects α–thalassemia-1 carriers resulting from other alpha thalassemia mutations that spare the embryonic zeta globin genes and causes traces of zeta-peptide to persist throughout life. DAI Zeta Globin Assay does not detect alpha thalassemia carriers and traits that do not result from the deletion. These include heterozygous α–thal-2 (α-/αα ) and homozygous α–thal-2 (-α/α) and deletion. For diagnostic purpose, globin values should be used as an adjunct to other data available to the physician.

PERFORMANCE CHARACTERISTICS
PRECISION
Intara-Assay Variation: Intra assay variation was determined by assaying 3 specimens: Negative, mid-level positive and high positive) of 8 in a single run. The intra-assay coefficients of variations (CV’s) were 5.99%, 8.36% and 7.74% for the negative, mid-level positive and high positive respectively.
Inter-Assay Variation: Inter-assay CV’s were 7.53%, 12.49% and 8.52% for the negative, mid-level positive and high positive respectively in duplicate in 6 different runs for 3 days.

INTERFERENCES: Samples with HbE do not interfere in the ZAM kit.
COMPARISON STUDY
A total of 161 blood samples obtained from patients reported to physicians with clinical signs of symptoms related to thalassemia were evaluated. The results from DAI Zeta Globin Assay were compared to the results of PCR/DNA method to delete the –gene mutation.

<table>
<thead>
<tr>
<th>Result</th>
<th>DAI ZETA GLOBIN</th>
<th>PCR/DNA METHOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>78</td>
<td>78</td>
</tr>
<tr>
<td>Negative</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>Total</td>
<td>161</td>
<td>161</td>
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</tbody>
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SPECIFICITY: 100%
SENSITIVITY: 100%
ACCURACY: 100%

REFERENCE