DRG® Zeta Globin Elisa (EIA-5047)

RUO

As of 28 May 2009 (Vers. 1.1)

Please use only the valid version of the package insert provided with the kit

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 NAME AND INTENDED USE

The ZETA GLOBIN ASSAY 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*)

2 PRINCIPLE OF THE ASSAY

The Zeta Globin ELISA 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

3 WARNINGS AND PRECAUTIONS

1. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
2. 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.

4 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.

5 SAFETY INSTRUCTIONS

1. Negative and Positive Control are from 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. Nonacidic 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.

6 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** (11mL):
   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 HCI.
10. **Well holder**: For securing individual wells.

7 MATERIALS REQUIRED BUT NOT PROVIDED

1. Micro-well reader at 450 nm.
2. Pipetor with tips for 25 µL & 100 µL
3. Water bath or incubator with temperature control (37°C)

8 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 1 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.
Hemolysed blood sample is ideal for the assay.

9 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.

10 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 µL** of **sample diluent** into each test tube.
   Add **500 µL** of 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.
   Secure the desired number of coated wells in holder. Record the identification of each well
4. Transfer **100 µL** of the above **treated sample** (sample, reference standard or controls) to maintain specimen identification of each respective well in duplicate.
5. Incubate at **37°C for 30 minutes**.
   Invert wells to decant incubation mixture. Tap wells to ensure thorough removal of incubation mixture.
6. **Rinse** the wells **5 times** with wash buffer.
7. Dispense **100 µL** of **enzyme conjugate** into each well and mix for 5 seconds and incubate in **37°C for 30 minutes**.
8. 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).
9. Dispense **100 µL** of **Solution A** and **100 µL** 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)

10. Stop reaction by adding **50 µL** of 2 N **HCI solution** to each well
and **read** at 450 nm with microwell reader against Blank well (only Solution A and Solution B).

11 RESULTS

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

12 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.

13 APPLICATIONS & LIMITATIONS

The 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.
The 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.

14 REFERENCE

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3. Weatherall DJ, Cleff JB. The Thalassemia Syndromes (ed3): Blackwell, NewYork, NY, 1981.
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5. Sabath, DE et al, Analysis of human zeta-globin gene promoter in transgenic mice. Blood 82:2899-905, 1993.
6. Ireland, J.H. et al, detection of the Double alpha Globin gene Deletion by a Simple Immunological Assay for Embryonic Globin chains. Amer Jour of Hematology, 44:22-28, 1993