ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	**#0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1970

Human α-Thalassemia ($^{--SEA}$) ζ Globin (ZAM)

ELISA Kit Cat. #. 1970

For rapid screening for the determination of elevated Zeta (ζ) globin levels in whole blood to aid in the detection of α-thalassemia-1 carrier resulting (--SEA) deletion

For In Vitro Research Use Only

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Human α-Thalassemia ($^{(--SEA)}$) ζ Globin (ZAM)

Cat. #1970; Kit Contents: (reagents for 96 tests)

Components	96 tests
ζ peptide antibody coated microwell strip **plate** (96 wells), Cat. # 1971	1 plate
Negative Control (0.3 mL), Containing no (--SEA) blood #1972A	1 Vial
Positive Control (0.3 mL), Containing no (--SEA) blood #1972B	1 Vial
Sample Diluent (20 mL) Lysing reagent for Red blood cells #1973	1 bottle
Enzyme conjugate (11 mL): Anti-ζ Antibodies conjugated with peroxidase # 1974	1 bottle
Wash buffer (100X), 10 ml, Cat#W-100; dilute 1:100 with distilled water,	1 bottle
HRP substrate **Soln A**, Cat. #1970-SA, 11 ml	1 bottle
HRP substrate **Soln B**, Cat. #1970-SB, 11 ml	1 bottle
Stop solution, 10 ml, Cat. # T-10	1 bottle
Instruction Manual, M-1970	1

Alpha thalassemia is by far the most prevalent genetic disorder of humans. 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 (--SEA) mutations is the one that puts patients at greatest risk. The most common alphathalassemia mutation in Southeast Asia or Southern Chinese populations is the (--SEA) mutation double alpha-globin deletion.

Carriers of (--SEA) 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. The (--SEA) deletion spares the embryonic zeta globin genes and carries traces of zetapeptide to persist throughout life. Hence in (--SEA) 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. Zeta globin is the embryonic form of the alpha chain of hemoglobin. Zeta globin chains that can be detected by antibodies provide rapid, simple, and reliable screening for the (--SEA) double alpha- globin deletion.

ADI's is a solid phase enzyme linked immunosorbent assay. This test provides rapid screening for the determination of elevated Zeta (ζ) globin levels in whole blood to aid in the detection of alpha-thalassemia-1 carrier resulting (--SEA) deletion.

References

Harada F (1994) Med. Metabol. Bio. 51, 80-84; Chui DHK (1989) Blood 74, 1409; Sabath DE (1993) Blood 82, 2899; Ireland JH (1993) J. Hematol. 44, 22-28

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

ADI Zeta Globin Assay detects ⟨-thal-1 carriers resulting from the (--SEA/) deletion. It also detects ⟨-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. ADI Zeta Globin Assay does not detect alpha thalassemia carriers and traits that do not result from the (--SEA) deletion. These include heterozygous ⟨-thal-2 (⟨□/⟨⟨) and homozygous ⟨□thal-2 (□⟨/□⟨) and (--TOT/) deletion. For diagnostic purpose, ζ globin values should be used as an adjunct to other data available to the physician.

PERFORMANCE CHARACTERISTICS

PRECISION

Intra-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 intraassay 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 ADI Zeta Globin Assay were compared to the results of PCR/DNA method to delete the –SEA/gene mutation.

Results	ADI ELISA kit	PCR/DNA method
Positive	78	78
Negative	83	83
Total	161	161

SPECIFICITY: 100% **SENSITIVITY**: 100% **ACCURACY**: 100%

PRINCIPLE OF THE TEST

ADI ζ Globin Assay is a solid phase enzyme linked immunosorbent system employing plastic wells coated with ζ 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 solutions are added. The color developed indicates the presence z peptide in the sample, a solid phase enzyme linked immunosorbent assay.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-100 ul) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader, 37oC incubator

PRECAUTIONS

The Alpha Diagnostic Intl., Inc. NSE ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN 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. Hemolysed blood sample is ideal for the assay.

Preparation of reagents

Wash buffer is supplied as **100x stock. Dilute 10 ml** into 1 litre de-ionized or distilled water, mix, and store at room temp for 1-2 weeks. It can be stored at 4oC for long term storage.

All other reagents are supplied ready to use.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping. Standards are stable for two month at 2-8^{o}C. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).).* **Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter).**

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. **Sample extraction/Lysis step**. Secure the appropriate # of glass tube (12x17mm) and label appropriately. Dispense **200 ul sample diluent** into all tubes. Add **40 ul of standards**, controls or samples into appropriate wells in *duplicate*. Vortex each tube vigorously for 20 seconds to make sure that red blood cells lyse completely. Set these aside until step 2.

2. Label or mark the microtiter well strips supplied in the kit to be used on the plate. **Transfer 100 ul of standards, controls, and samples** as treated in step 1 above in duplicate. You must maintain the sample identification from the tubes to the wells (step 1 to 2). **Incubate at 37oC for 30 min**.

3. Wash the plate **5X** with wash buffer (300 ul/wash). We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing

4. Add **100 ul antibody-enzyme conjugate** into each well**.** Mix gently for 5 seconds, cover the plate and incubate for **30 minutes** at 37oC.

5. Wash the plate **5X** with wash buffer (300 ul/wash).

6. Premix substrate **solution A and B** into 1:1 ratio (1 ml of A and 1 ml of B) and dispense **200 ul of the substrate mix into all wells.** Mix gently for 5 seconds and **incubate** in the dark for **15 min** at room temp. Notes: Substrate solution must be at room temp prior to the addition into the plate. Prepare the mix as needed. For a full plate assay, prepare 20 ml substrate mix (10 ml of A and 10 ml B).

7. Stop the reaction by adding **50 ul of stop solution** into all wells. Mix gently. Blue color turns yellow. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min.

NOTES-

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C.

Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

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 ADI ZAM Assay.

Positive: Samples that developed the color equal to or stronger than 0.3 A.U. are considered Positive.