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

3100	Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3105	Human anti-dsDNA IgM ELISA Kit, 96 tests, Quantitative
3110	Human anti-dsDNA IgA ELISA Kit, 96 tests, Quantitative
3115	Human anti-ssDNA IgG ELISA Kit, 96 tests, Quantitative
3205	Human Anti-Nuclear Antibodies (ANA) ELISA Kit, 96 tests, Semi-
3210-SSA	Human anti-SS-A/Ro IgG ELISA Kit, 96 tests, Quantitative
3220-SSB	Human anti-SS-B/La IgG ELISA Kit, 96 tests, Quantitative
3250	Human Anti-thyroid peroxidase ELISA kit, Semi-Quantitative
3300	Human Anti-helicobacter pylori IgG ELISA kit, Semi-Quantitative
3300-100-SMG	Human Anti-Smith antigen (Sm) IgG ELISA kit, 96 tests, Quantitative
3300-110-SRG	Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit, 96 tests,
3300-120-RNG	Human Anti-RNP (RNP-70) IgG ELISA kit, 96 tests, Quantitative
3300-130-HNG	Human Anti-histones IgG ELISA kit, 96 tests, Quantitative
3300-140-SCG	Human Anti-Scl-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-150-JOG	Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-160-AFG	Human Anti-Alpha Fodrin IgG ELISA kit, 96 tests, Quantitative
3300-170-CLG	Human Anti-Cardiolipin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM	Human Anti-Cardiolipin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA	Human Anti-Cardiolipin IgA ELISA kit, 96 tests, Quantitative
3300-190-B2G	Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-195-B2M	Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative
3300-200-APS	Human Anti-Phospholipid Screen IgG/IgM ELISA kit, 96 tests,
3300-200-B2A	Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-210-PSS	Human Anti-Phosphotidyl serine IgG/IgM ELISA kit, 96 tests,
3300-215-PIS	Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests,
3300-220-PAS	Human Anti-Phosphotidic Acid IgG/IgM ELISA kit, 96 tests,
3300-230-APG	Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests, Quantitative
3300-235-APA	Human Anti-Prothrombin IgA ELISA kit, 96 tests, Quantitative
3300-240-AVA	Human Anti-Annexin V IgG ELISA kit, 96 tests, Quantitative
3300-250-ANG	Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit, 96
3300-255-PRG	Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG	Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative
3300-270-GBG	Human Anti-glomerular basement membrane (GBM) IgG ELISA kit, 96
3300-280-BPG	Human Anti-bactericidal permeability increasing (BPI) protein IgG
3300-290-ELG	Human Anti-Elastase IgG ELISA kit, 96 tests, Quantitative
3300-300-GLG	Human Anti-Gliadin IgG ELISA kit, 96 tests, Quantitative
3300-305-GLM	Human Anti-Gliadin IgM ELISA kit, 96 tests, Quantitative
3300-310-GLA	Human Anti-Gliadin IgA ELISA kit, 96 tests, Quantitative
3300-315-PRG	Human Anti-Parietal cell (alpha and beta subunits of the Parietal Cell (H//K/ATPase) IgG ELISA kit, 96 tests,
3300-320-ASC	Human Anti-ASCA (mannan from Saccharomyces cerevisiae) IgA/IgG
3300-330-ASG	Human Anti-Sperm IgG ELISA kit, 96 tests, Quantitative
3300-340-CCG	Human Anti-Cyclic Citrullinated Peptide (CCP)IgG ELISA kit, 96 tests,
3300-350-TPG	Human Anti-thyroid peroxidase (TPO) IgG ELISA kit, 96 tests,
3300-360-TGG	Human Anti-thyroglobulin (TG) IgG ELISA kit, 96 tests, Quantitative
3310	Human Anti-helicobacter pylori IgM ELISA kit, Semi-Quantitative
3320	Human Anti-helicobacter pylori IgA ELISA kit, Semi-Quantitative
3600-HIG	Human Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3610-MKG	Monkey Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3700-MIG	Mouse Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3710-MIM	Mouse Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
3750-RIG	Rat Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3760-RIM	Rat Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
4000	Mouse Anti-Myelin Oligodendrocytes protein (MOG35-55) Ig's ELISA kit,

Monkey Anti-Insulin IgG (Autoantibodies)

ELISA KIT Cat. # 3610-MKG

For the Quantitative Determination of Autoantibodies to Insulin in monkey serum or plasma

For In Vitro Research Use Only

India Contact:

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Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@atzlabs.com
Web: www.atzlabs.com

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Monkey Anti-Insulin ELISA KIT Cat. # 3610-MKG (96 tests)

Kit Components (96 tests)	Cat #
Recombinant Insulin coated strip plate, (8x12 strip or 96 wells) # 3611	1 plate
Anti-Insulin Calibrator A (0 u/ml, 1.5mL) #3612A	1 vial
Anti-Insulin Calibrator B (6.3 u/ml, 1.5mL) #) #3612B	1 vial
Anti-Insulin Calibrator C (12.5 u/ml, 1.5mL) #) #3612C	1 vial
Anti-Insulin Calibrator D (25 u/ml, 1.5mL) #) #3612D	1 vial
Anti-Insulin Calibrator E (50 u/ml, 1.5mL) #) #3612E	1 vial
Anti-Insulin Calibrator F (100 u/ml, 1.5mL) #) #3612F	1 vial
Anti-Insulin Controls in Matrix (+ve & -ve) #) #3613P and 3613N, 1.5 ml each (lot sp values are provided on the vials)	2x 1 vial
All controls contain 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane as preservative	1 vial
Anti-mk-IgG-HRP Conjugate, (15 ml) ##3614 (red soln)	1 bottle
Sample Buffer (5X), 20 ml # 3615 (yellow soln)	1 bottle
Wash buffer (50X), 20 ml # 3610-WB	1 bottle
TMB Substrate Solution, 15 ml #3610-SS	1 bottle
Stop Solution, 15 ml # 3610-ST	1 bottle
Plastic foils (2) for covering plates and Resealable bag	1 bottle
Complete Instruction Manual	M-3610-MKG

INTENDED USE

ADI's Anti-Insulin ELISA Test Kit has been designed for the quantitative determination of Autoantibodies to Human/monkey Insulin in serum and plasma. The assay is useful for the determination of Type I Diabetes, as well as for screening purposes to detect developing insulin autoantibodies in monkey under insulin therapy.

INTRODUCTION

Type I Diabetes is mainly characterized by limited or fully missing secretion of the hormone insulin. Morphological studies demonstrated a destruction of the beta cells of the so-called Langerhanns's Cells (Islet Cells) in Type I diabetics. Numerous researchers described the appearance of antibodies directed against the islet cells and insulin as the causal reason for the onset of the disease. Anti-Insulin antibodies are found in 37 percent of patients with newly detected Type I Diabetes, in 4% of their relatives of the first degree and in up to 1.5% of healthy controls. A positive correlation between the appearance of anti-Insulin and anti-Islet Cell antibodies has been reported. Anti-Insulin autoantibodies may be detected several months and in some cases years before the onset of the fully clinical manifestation of the diseases. Occasionally also autoantibodies to Pro-Insulin may appear. These "true" anti-Insulin autoantibodies directed against endogenous insulin have to be distinguished from those autoantibodies which are developed in insulin dependent diabetics undergoing therapy with insulin preparations of animal origin. In fact the latter have to be referred to side effects. These side effects may occur as local reactions of the skin by development of insulin-specific autoantibodies. These autoantibodies are causing the formation of an insulin depot and they may simulate a resistance against the hormonal treatment with animal insulin. Additionally other immunological phenomenon have been

NORMAL VALUES

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the anti-insulin test:

		Anti-Insulin (U/mL)			
Normal	< 5	**Borderline**	5-10	**Elevated**	>10

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges. The reference ranges below should be regarded as guidelines only. **Note**: These data is derived from human studies since similar data for monkey samples is not available.

A study of 16 random samples of cynomolgous, rhesus, baboons sera produced anti-insulin IgG levels close to the background (A450=0.100-0.200 or 0 U/ml).

Quality Control

The test results are only valid if the test has been performed following the instructions. All standards and kit controls must be found within the acceptable ranges as stated on the vials. The positive control must show at least double the OD of the cut-off standard. If criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven (reagents, protocol, equipments, etc).

PERFORMANCE CHARACTERISTICS

Intra-Assay-Precision: 2.5-4% **Inter-Assay-Precision:** 4-6 %

Sensitivity: 0.5 U/mL **Range:** 6.3-100 U/mL

Parallelism

Two samples with an initial anti-Insulin IgG concn of 77.6 and 110.7 u/ml were diluted 1:50; 1:100, 1:200, 1:400, 1:800 and re-assayed in the ELISA kit. Recovery was85-107%.

SPECIFICITY and Animal Crossreactivity

The microplate is coated with a mixture of highly purified preparations of recombinant human insulin (monkey insulin is the same). The conjugate used in the kit is against the monkey IgG, therefore, only IgG-subclass antibodies will be detected. However, most anti-monkey IgG are highly cross-reactive with human Ig's. Therefore, this kit may not distinguish between human and monkey antibodies if present in the same sample. ADI has separate anti-insulin ELISA kits for mouse and rat samples.

Standard Unit Calibration

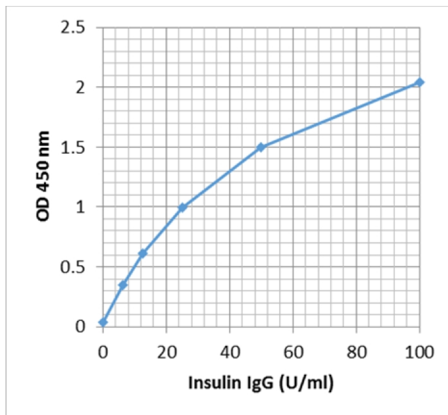
No international reference preparation for monkey anti-Insulin autoantibodies is available, the assay system is calibrated in relative arbitrary units.

References: Jin, X (2010) Exp. Biol. Med. 235, 877-885; Koulmanda M (2003) Am. J. Transplant. 3, 267-272; Theriault BR (1999) Transplantation. 68:331-7 Wagner, JD (2001) Toxicol. Pathol. 29, 142-148; Reeves, W.G. et al (1983) Diabetologia, 24, 399; Atkinson, M.A., (1985) Diabetes 34, 926-930; Willein, T. (1985) Diabetes 76, 185; Kobayashi, N.(1985) J. Immunol. Methods 84; 245. Wissllein, T (1985) Lancet 1, 480; Soeldner, J.S. (1985) NEJM 313, 893.

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Average net A450	Net A450	Results
A1, A2	**Blank**	0.100		
B1, B2	Standard A (0 U/ml)	0.103	0.034	
C1, C2	Standard B (6.3 U/ml)	0.449	0.349	
D1, D2	Standard C (12.5 U/ml)	0.711	0.611	
E1, E2	Standard D (25 U/ml)	1.094	0.994	
F1, F2	Standard E (50 U/ml)	1.602	1.502	
G1, G2	Standard F (100 U/ml)	2.146	2.046	
H1, H2	Sample 1	1.100	1.00	25 U/ml

NOTE: These data are for demonstration purpose only. It must not be used to determine the sample results.



/4_ADI-Graph

CALCULATION OF RESULTS

1. First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

2. Obtain the values of samples from the standard curve.

3. Multiply the sample values only if the samples were diluted more than 1:100 (e.g., if sample diluted by 1:500 then multiply the values by 5).

reported for Type I diabetics. A lot of other autoantibody specificities have been detected in those patients too, but these antibodies must not cause additional autoimmune phenomenon. The table below shows the incidence (in percent) of various autoantibodies occurring in Type I diabetics compared to healthy controls:

	Type 1 diabetes (%)	Healthy Controls (%)
Anti-Islet Cell antibodies	32	1
Anti-Islet Cell surface antigens	~50	~2
Anti-Insulin antibodies	up to 70	0
Anti-Thyroid peroxidase IgG	8	6
Anti-ssDNA)	85	9

Note: These data is derived from human studies since similar data for monkey samples is not available.

Diabetes occurs naturally in monkeys and results in changes in plasma lipids and lipoproteins similar to those that occur in human diabetics. A number of reports have described successful methods for inducing insulin-dependent diabetes in nonhuman primates. These methods include total pancreatectomy or treatment with streptozotocin (STZ) at a variety of different doses. Streptozotocin, derived from Streptomyces achromogenes, is toxic to beta cells. Monkey may also develop autoantibodies to insulin as reported in humans.

Insulin is made up of 51 amino acids and is one of the smallest proteins in the body. It is comprised of two polypeptide chains (A and B) linked by two disulfide bonds, connecting cysteine molecules. The amino acid sequence of insulin is highly conserved among vertebrates. Virtually all primates and monkeys share the same sequence. Human insulin A and B chains are 100% conserved in great apes and cynomolgous monkey. Rat and mouse insulins only differ from primate insulin by 4 amino acids. Thus, insulin from one species is often biologically active in other species. For example, pig insulin has historically been used to treat human patients. Insulin from some species of fish can also be similar enough to human insulin to be clinically effective in humans. In addition, insulin in some invertebrates is quite similar to human insulin, and has similar physiological effects.

PRINCIPLE OF THE TEST

ADI's Anti-Insulin IgG antibody test kit is based on the principle of the indirect ELISA. Insulin antigen is bound on the surface of the microtiter strips. Diluted samples (serum or plasma) or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized antigen takes place. After an incubation, the plate is rinsed with diluted wash solution, in order to remove unbound material. Bound insulin antibodies are detected by anti-IgG peroxidase conjugate (species and isotype specific). After a further washing step, the substrate (TMB) solution is added for the development of a blue color in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The yellow color is measured at 450 nm using an ELISA reader. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipette (10µl, 100µl, 1000µl) and multichannel pipet with disposable plastic tips, distilled water, reagent troughs, graduated cylinder for 100 & 1000mL, plastic container for storage of wash solution, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

PRECAUTIONS

Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision.

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).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

For determination of anti-Insulin antibodies serum or plasma are the preferred sample matrixes. All serum and plasma samples are prediluted 1:100 with sample buffer. Therefore 10 ul of sample may be diluted with 1,000 µl of sample buffer. The patients need not to be fasting, and no special preparations are necessary. Collect blood by venipuncture into vacutainers and separate serum or plasma from the cells by centrifugation after clot formation. Samples may be stored refrigerated at 2 - 8 °C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20 °C. To avoid repeated thawing and freezing the samples should be aliquoted. Neither Bilirubin nor Hemolysis have significant effect on the procedure.

REAGENTS PREPARATION

1. **Dilute Wash Buffer**: Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2 - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

2. **Dilute Sample Buffer:** Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2 - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8°C or stored frozen in small aliquots and should be stable for 3 months.

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°C. Do not touch the bottom of the wells.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Dilute the wash buffer and sample buffers with water (see page 3). Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. **All samples should be diluted 1:100 (10 ul samples in 1000 ul sample buffer)**. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate. **Calibrators and controls are ready to use and need not to be diluted**.

1. Label or mark the microtiter well strips to be used on the plate.

2. Dispense **100 ul of calibrators, controls, and diluted patient samples** (1:100 or higher) into appropriate wells in *duplicate*. Cover the plate, mix gently for 5-seconds and incubate at room temp for 30 min.

3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 300 ul of wash buffer. 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 Anti-IgG-HRP conjugate** to all wells. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temp (25-28oC).

5. Wash the wells 3 times as in step 3.

6. Add **100 ul TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temperature protected from light.

7. Stop the reaction by adding **100 ul of stop solution** to all wells and leave untouched for 5 minutes. Mix gently for 5-10 seconds to have uniform color distribution.

8. Measure the **absorbance at 450 nm** using an ELISA reader within 30 minutes. Bi-chromatic measurement with reference at 600-650 nm is recommended.