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

990-100-THA	Human Anti-Mycobacterium Tuberculosis IgA ELISA kit, 96 tests
990-110-THG	Human Anti-Mycobacterium Tuberculosis IgG ELISA kit, 96 tests
990-120-THM	Human Anti-Mycobacterium Tuberculosis IgM ELISA kit, 96 tests
990-210-TMG	Mouse Anti-Mycobacterium Tuberculosis IgG ELISA kit, 96 tests
990-220-TMM	Mouse Anti-Mycobacterium Tuberculosis IgM ELISA kit, 96 tests
990-230-06G	Mouse Anti-M. Tuberculosis 6kDa/ESAT-6 IgG ELISA kit, , 96 tests
990-235-06M	Mouse Anti-M. Tuberculosis 6kDa/ESAT-6 IgM ELISA kit, , 96 tests
990-240-16G	Mouse Anti-M. Tuberculosis 16kDa/Hspx IgG ELISA kit, , 96 tests
990-245-16M	Mouse Anti-M. Tuberculosis 16kDa/Hspx IgM ELISA kit, 96 tests
990-250-38G	Mouse Anti-M. Tuberculosis 38kda/Ag85b IgG ELISA kit, , 96 tests
990-255-38M	Mouse Anti-M. Tuberculosis 38kda/Ag85b IgM ELISA kit, , 96 tests
990-260-38G	Human Anti-M. Tuberculosis MVA vaccine (38kda/Ag85b) IgG ELISA
990-265-38M	Human Anti-M. Tuberculosis MVA vaccine (38kda/Ag85b) IgM ELISA
990-310-TRG	Rabbit Anti-Mycobacterium Tuberculosis IgG ELISA kit, 96 tests
990-320-TRM	Rabbit Anti-Mycobacterium Tuberculosis IgM ELISA kit, 96 tests
990-400-MTG	Monkey Mycobacterium Tuberculosis IgG ELISA kit, 96 tests, 990-
990-410-MTM	Monkey Mycobacterium Tuberculosis IgM ELISA kit, 96 tests

960-110-PHG	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG, 96 tests,
960-120-PHG	Mouse Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG ELISA kit,
960-130-PMG	Mouse Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative
960-140-PMM	Mouse Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative
960-150-PRG	Rabbit Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative
960-160-PRM	Rabbit Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative
960-170-PMG	G. pig Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative
960-180-PMM	G. pig Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative
960-200-PHA	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA kit,
960-205-PHA	Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA kit,
960-210-PHG	Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG ELISA kit,
960-220-PHM	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit,
960-225-PHM	Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit,
960-230-PGG	Mouse Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-240-PRG	Rabbit Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-250-PHG	Human Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-260-PMG	Monkey Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-300-FMG	Mouse Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit, 96
960-310-FMM	Mouse Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit, 96
960-320-FRG	Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit, 96
960-330-FRM	Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit, 96
960-340-FHG	Human Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit, 96
960-350-FHM	Human Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit, 96

940-100-DHG	Human Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-120-DMG	Mouse Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-125-DMM	Mouse Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
940-130-DRG	Rabbit Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-135-DRM	Rabbit Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
940-140-DGG	Guinea Pig Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-145-DGM	Guinea Pig Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
940-150-HFA	Horse Anti-Diphtheria Toxin/Toxoid IgG (Fab2) ELISA kit, 96 tests, Quantitative
940-200-DHG	Human Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests,
940-210-DHM	Human Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests,
940-220-DMG	Mouse Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests,
940-225-DMM	Mouse Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests,
940-230-DRG	Rabbit Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests,
940-235-DRM	Rabbit Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests,
940-245-DKM	Monkey Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative

Instruction Manual No. M-990-110-THG

Human Anti-Mycobacterium Tuberculosis IgG ELISA Kit

Cat. # 990-110-THG

For the detection of IgG class antibodies against M. tuberculosis in serum and plasma

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
Ph: +91-11-42208000, 42208111, 42208222

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

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

Human Anti Mycobacterium Tuberculosis IgG ELISA KIT #990-110-THG

Kit Components	Qty
Recombinant purified M. tuberculosis antigens (18, 36, 40 kda) coated **microwell strips** (96 wells); #990111	1 Plate
Calibrator A, Negative Control, 1.5 ml ;# 990112A	1 vial
Calibrator B, Cut-off Control, 1.5 ml,# 990112B	1 vial
Calibrator C, Weak-positive Control, 1.5 ml,# 990112C	1 vial
Calibrator D, Positive Control, 1.5 ml ,# 990112D	1 vial
Controls are diluted serum base containing 0.01% BND as preservative	
Sample Diluent, 60 ml BSA containing buffer with 0.09% azide, ready to use; #990110-SD	1 bottle
Wash buffer (10X), 60 ml; #990110-WB	1 bottle
Anti-human IgG **HRP Conjugate**, 15 ml, #990113	1 bottle
HRP Substrate Soln (TMB) , 15 ml, #990110-TMB	1 bottle
Stop Solution (diluted sulfuric acid), 15 ml; #990110-ST	1 bottle
Complete Instruction Manual; M-990-110-THG	

Intended Use

Mycobacterium tuberculosis IgG antibody ELISA kit has been designed for the detection of IgG class antibodies against Mycobacterium tuberculosis in serum and plasma. For research use only, not for use in diagnostic procedures.

General Information

Mycobacterioses (tuberculosis, leprosy, atypical mycobacterioses, paratuberculosis, and perhaps Crohn's Disease) are diseases of men and animals with the largest diffusion on earth. The infectious agents of tuberculosis are acid-resistant rod-like bacteria of the family Mycobacteriaceae, genus Mycobacterium. The organism was detected by Robert Koch in 1882. Owing to the very high infectivity of pathogenic mycobacteria, early diagnosis is essential to prevent spreading of the disease. One third of the world's population is thought to have been infected with M. tuberculosis, with new infections occurring at a rate of about one per second. TB killed 1.4 million people in 2010.

The main cause of TB is Mycobacterium tuberculosis, a small, aerobic, nonmotile bacillus. The high lipid content of this pathogen accounts for many of its unique clinical characteristics. If a Gram stain is performed, MTB either stains very weakly "Gram-positive" or does not retain dye as a result of the high lipid and mycolic acid content of its cell wall. The only currently available vaccine as of 2012 is **bacillus Calmette–Guérin** (**BCG** with live attenuated bacteria) which, while it is effective against disseminated disease in childhood, confers inconsistent protection against contracting pulmonary TB.

Interpretation of results

Most of the data presented here for information purpose. Therefore, users are suggested to establish their own reference values.

1. Evaluation of results is easily carried out by direct comparison of the optical density of each sample with the optical density of the cut-off control (B). Samples exhibiting optical densities higher than the optical density of the cut-off control are considered to be positive.

Negative:	<8 U/ml
Equivocal	8-12 U/ml
Positive	>12 U/ml

Expected Values

An in-house study of normal human random samples showed the following results.

Ig isotype	n	Interpretation		
		Positive	Equivocal	Negative
IgG	88	1.1%	0%	98.9%

PERFORMANCE CHARAVEREISTICS

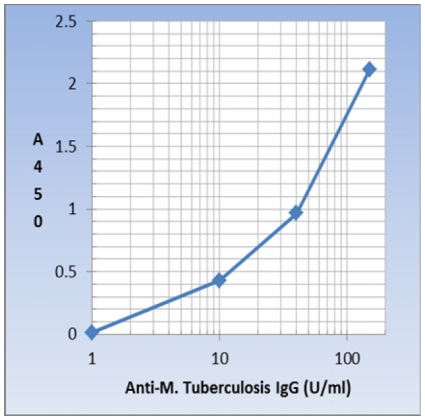
Mycobacterium ELISA IgG	
Intra-Assay-Precision	7.6 %
Inter-Assay-Precision	9.4 %
Inter-Lot-Precision	3.1 – 9.9 %
Analytical Sensitivity	1.09 U/mL
Recovery	86 – 95 %
Linearity	82 – 113 %
Cross-Reactivity	No cross-reactivity to Helicobacter pylori and Bordetella pertussis.
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL
Clinical Specificity	99 %
Clinical Sensitivity	100 %

References: Bloom R (1992) Science 257, 1055-1064; Snider DE (1994) J. Infec. Dis. 169, 1189-1196;

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (U/ml)	Net Mean $A_{450\ nm}$	Calculated Conc. (U/ml)
A1, A2	**Std. A** negative control (1 U/ml)	0.016	
B1, B2	**Std. B** Cut-off (10 U/ml)	0.439	
C1, C2	**Std. C** Weak positive control (40 U/ml)	0.967	
D1, D2	**Std. D** positive control (150 U/ml)	2.11	
F1, F2	**Sample 1**	0.95	40.01

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



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INTERPRETAION AND CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log.For the calculation of the standard curve apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).The concentration of the samples can be read from the standards curve. The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor. Samples showing concentrations above the highest standard have to be diluted as described in “Assay

Nevertheless, it is the most widely used vaccine worldwide, with more than 90% of all children being vaccinated. The BCG vaccine can be anywhere from 0 to 80% effective in preventing tuberculosis for a duration of 15 years; however, its protective effect appears to vary according to geography and the lab in which the vaccine strain was grown.

A number of new **TB vaccines** are currently in phase I and II clinical trials. MVA85A (modified vaccinia Ankara 85A, Oxford University) is a subunit vaccine to BCG. This vaccine produces higher levels of long-lasting cellular immunity when used together with the old TB vaccine called BCG. It uses the attenuated MVA as a vaccine delivery platform to present antigen 85A to the immune system.

BCG vaccines: Pacis® BCG, made from the Montréal (Institut Armand-Frappier) strain (Dianon/Urocor). BCG vaccine Danish strain 1331 (Statens Serum Institut, Denmark), Tokyo BCG TY-1002, Tokyo 172 substrain of Pasteur BCG (Japan, BCG Labs), Moscow BCG 254-2; BCG vaccine Glaxo 1077 strain (Sanofi). All vaccine use attenuated M. Bovis strains.

PRINCIPLE OF THE TEST

Anti-M. tuberculosis IgG) ELISA kit is based on binding of antibody from serum samples to M. tuberculosis antigens immobilized on microtiter wells. After a washing step, anti-IgG-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of IgG present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of IgG in samples is calculated compared with the absorbance of the supplied negative and positive controls.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

This ELISA test is intended for *in vitro research* use only. The reagents contains human serum and preservative; necessary care should be taken when disposing solutions. Human sera are 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 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

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20^{O}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. EDTA/Heparin plasma can also be used.

Preparation of the reagent:

Dilute wash buffer (1:10) with distilled water (**60 ml stock in total of 600 ml water**). **s**tore at 4oC. If stock shows crystal then it can be dissolved by bringing to room temp or slight warming.

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 12 months from the date of shipping under appropriate storage conditions. Do not contaminate the bottles. Withdraw solutions in a separate clean tube or dispensing trays. Any unused solution should be discarded and not returned to the bottle. Do not use HRP substrate solution if this solution is blue. Do not expose these solutions to strong light.

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

1. Label, and secure the microtiter well strips to be used on the plate. **Dilute** samples (1:101) in sample diluent. Controls provided in the kit are already pre-diluted.

2. Pipet **100 ul of sample** diluent (for use as blanks), pre-*diluted* negative, positive controls, and *diluted* serum samples into appropriate wells in *duplicate*. Mix gently for 5-10 seconds, cover the plate and incubate for **60 minutes** at room temp (24-28oC).

3. Aspirate and **wash the wells 3 times** with 300 ul of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Add **100 ul of antibody-enzyme conjugate** into each well**.** Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp.

5. Aspirate and wash the wells 3 times as above.

6. Dispense **100 ul TMB substrate per well**. Mix gently for 5 seconds. Cover the plate and incubate at room temp in the dark. for **20 minutes**. **Blue color** develops in positive wells.

7. Stop the reaction by adding **100 ul** of stopping solution to all wells at the same timed intervals . Mix gently for 5-10 seconds. **Blue color turns yellow**. Measure the absorbance at 450 nm using an ELISA reader.

QUALITY CONTROL

This test is only valid if the optical density at 450 nm for negative control (NC), cut-off control (CC) and positive control (PC) complies with the respective range indicated in this manual or on the Quality Control Certificate enclosed with the kit. If any of these criteria is not fulfilled, the results are invalid and the test should be repeated. The assays is calibrated against the reference standards for human and internal monkey controls.

Each time the assay is run, the cutoff control must be run in triplicate. A positive and negative control must also be included in each assay.

The mean OD value for the cut-off and the OD values for positive and negative controls should fall within the following ranges:

	A450 (OD range))
Negative Control	<0.200
Cutoff control	>Negative control
Positive control	≥0.500

Additional controls may tested according to guidelines or requirement of local, state and/or federal regulations or accredited organizations.

Human and Animal specificity

This kit employs anti-IgG-HRP conjugate that reacts with human IgG with no significant detection of IgM or IgA. ADI has other kits to detect human IgG/IgA or mouse or rabbit anti-M. Tuberculosis antibodies.