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

Catalog#	ProdDescription
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
7050	Monkey IgG (total) ELISA Kit, 96 tests, Quantitative
7060	Monkey IgM ELISA Kit, 96 tests, Quantitative
7070	Monkey IgE ELISA Kit, 96 tests, Quantitative
7075	Chimp IgE ELISA Kit, 96 tests, Quantitative

Instruction Manual No. M-990-400-MTG

Monkey Anti-Mycobacterium Tuberculosis IgG ELISA Kit

Cat. # 990-400-MTG

For the detection of Anti-M. Tuberculosis IgG In Monkey Serum/Plasma

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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

Monkey Anti M. tuberculosis IgG ELISA KIT #990-400-MTG

This kit has been designed for the detection of IgG class antibodies against M. tuberculosis in monkey serum or plasma. For research use only, not for use in diagnostic procedures.

Kit Components	Cat.#
Recombinant purified M. tuberculosis antigens (18, 36, 40 kda) coated **microwell strips** (96 wells)	990401
Calibrator A, Negative Control, 2 ml , ready to use	990402A
Calibrator B, Cut-off Control, 2 ml , ready to use	990402B
Calibrator C, Weak-positive Control, 2 ml, ready to use	990402C
Calibrator D, Positive Control, 2 ml , ready to use	990402D
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	990400-SD
Wash buffer (10X), 60 ml	990400-WB
Anti-mkIgG **HRP Conjugate**, 15 ml, ready to use	990403
HRP Substrate Soln (TMB) , 15 ml, ready to use	990400-TM
Stop Solution (diluted sulfuric acid), 15 ml	990400-SS
Complete Instruction Manual	M-990400MTG

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. Convergence of various approaches are necessary to control the mycobacterioses, immune reactions and bacterial shedding being variable during the diseases. However, typical procedures were until recently unsatisfactory and did not allow differentiation among different mycobacterial species. Disease is normally transferred by droplets of saliva from infected persons. The target of the infection are mostly the lungs, but also other organs such as the brain, intestinal tract, bones, lymph nodes and kidneys can be afflicted. Tuberculosis is not only found in developing countries with 8 million of new infections yearly, but also in industrialized civilizations, as an actual disease with some thousands of cases yearly. Without treatment, the disease leads in 50% of the cases to death within less than two years. Clinical symptoms are fatigue, loss of weight, lack of appetite, light fever, nocturnal sweat and pain in the chest. Inidividuals with HIV are at risk for infection by tuberculosis due to their impaired immune system. A vaccination with living attenuated bacteria is possible (BCG = Bacille Calmette Guérin). This is mostly done with newborn or young children. With older individuals, prior to vaccination the tuberculin test (Pirquet or Mantoux) is administered, where a small amount of tuberculin is injected under the skin. In a positive case, there exist antibodies against Mycobacteria, and a vaccination is not necessary. Up to recently, there have not existed any serological methods to detect tuberculosis antibodies in serum. The only available procedure in addition to the skin tuberculin test was direct microscopic

Interpretation of results

Most of the data presented here is derived from human studies and it is only being given for information purpose. No systematic large scale study has been undertaken in monkey population. Therefore, users are suggested to establish their own reference values for their animal population.

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.

Samples	Interpretation		
	Positive	Equivocal	Negative
88	1.1%	0%	98.9%

PERFORMANCE CHARAVEREISTICS

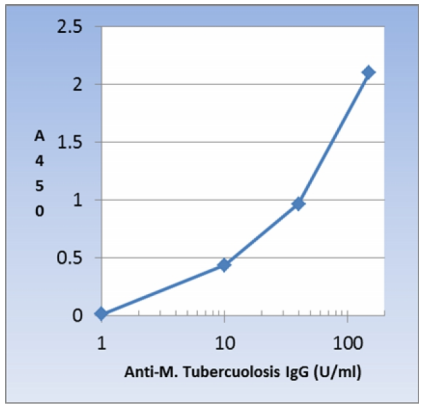
Mycobacterium ELISA IgG	
Intra-Assay-Precision	7.6 %
Inter-Assay-Precision	9.4 %
Inter-Lot-Precision	3.1 – 9.9 %
Analytical Sensitivity	1.09 U/mL
Recovery	86 – 95 %
Linearity	82 – 113 %
Cross-Reactivity	No cross-reactivity to Helicobacter pylori and Bordetella pertussis.
Interferences	No interferences 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)	0.017	
B1, B2	**Std. B** Cut-off (10 U/ml)	0.440	
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**	1.110	positive

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.



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

identification of the dyed bacteria in sputum. Recently specific antigens have been prepared either by purification of natural material or by recombinant methods.

Additional ELISA kits to detect the Mycobacterium tuberculosis virus antibody in mouse and other species are also available for research. These kits should be useful to determine the M. tuberculosis antibodies due to natural infection or upon vaccination with BCG vaccine.

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 or the web site.

TMB (substrate), H2SO4 (stop solution), and BND (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 (diluted sulfuric acid).

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^{o}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 (1:100) 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 4 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 (monkey specificity)

This kit employs anti-IgG-HRP conjugate that reacts with monkey (Rhesus, Cynomolgous, Chimp etc). The conjugate also react with human IgG. However, the antibodies are specific for IgG with no significant detection of IgM or IgA. ADI has other similar kits to detect anti-M. Tuberculosis IgM or IgA in monkey and humans.

Sensitivity

Anti-monkey IgG-HRP conjugate used in this kit detected control monkey IgG at 30 ng/well or 300 ng/ml.