

**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/Hsp60 IgG ELISA kit, , 96 tests  
990-245-16M Mouse Anti-M. Tuberculosis 16kDa/Hsp60 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 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-990-220-TMM

**Mouse Anti-Tuberculosis (Bacillus Calmette-Guérin/BCG; M. Bovis/M. Tuberculosis) IgM ELISA Kit**

**Cat. # 990-220-TMM**

For the detection of IgM class antibodies against BCG (M. Bovis/M. Tuberculosis/) in serum and plasma



*For In Vitro Research Use Only*



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**Mouse Anti-Tuberculosis (Bacillus Calmette–Guérin/BCG; M. Bovis/M. Tuberculosis)  
IgM ELISA Kit #990-220-TMM**

Kit Components	Qty
Purified BCG (M. Bovis, vaccine grade) antigens coated microwell strips (96 wells); #990211	1 Plate
Anti-BCG IgM std. A (1 U/ml), 1.0 ml ;# 990222A	1 vial
Anti-BCG IgM std. B (3 U/ml), 1.0 ml ;# 990222B	1 vial
Anti-BCG IgM std. C (10 U/ml), 1.0 ml ;# 990222C	1 vial
Anti-BCG IgM std. D (30 U/ml), 1.0 ml ;# 990222D	1 vial
Mouse Anti-BCG IgM Positive Control, 1.0 ml ,# 990223PC	1 vial
Standards and Controls contain buffer and 0.01% BND as preservative	
Anti-Mouse IgM-HRP Conjugate, 10X, 1.1 ml #RMC2-1	1 vial
Sample Diluent, 20X, 10 ml #SD-20T	1 bottle
Wash buffer (100X) 10 ml #WB-100	1 bottle
HRP Substrate TMB Substrate Solution, 12 ml #80091	1 bottle
Stop Solution, 12 ml # 80101	1 bottle
Complete Instruction Manual	M-990-220 -TMG

**Intended Use**

Mouse Anti-Tuberculosis (Bacillus Calmette–Guérin/BCG; M. Bovis/M. Tuberculosis) IgM ELISA kit is designed for the detection and measurement of IgM class antibodies against BCG tuberculosis antigen (*M. Bovis/M. Tuberculosis*) in serum and plasma of vaccinated or naturally infected animals. BCG antigens prepared from various strains of mycobacterium (*M. Bovis*, *M. Tuberculosis*, *M. Avium*, and *M. butyricum*) are antigenically similar. Therefore, this kit will detect BCG antibodies to various strains of mycobacterium in mouse samples. For research use only (RUO), 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

**Interpretation of results**

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

All samples when tested at 1:100 dilution with A450 value less than the Std B can be considered as negative or below the cut-off. All samples higher than the Std. B are positive of BCG IgGs.

Inbred strains have been used extensively to research the virulence mechanisms, pathology, and immune response associated with tuberculosis (TB) infections. Mouse inbred strains present with a wide spectrum of susceptibility/resistance to *Mycobacterium tuberculosis* infection.

**\*Strains susceptible to TB infection**

C57BL/6j (C57 Black; B6; B6J; Black 6), BALB/cJ, C57BL/10J, C3HeB/FeJ, SWR/J

**\*Strains resistant to TB infection**

DBA/2J, NOD/LtJ, 129X1/SvJ, A/J, C57L/J, CBA/J, NZB/BINJ, SJL/J J

\*Mouse strains available from Charles Rive or other suppliers.

**Mouse Sample Data**

A population of healthy, non-vaccinated young mice (Balb/C strain; n=8) were tested at 1:100. All mice showed A450 of less than the Std A or <0.300. Some mice (non-vaccinated) showed high concentration of BCG IgG (1:100 dilution A450 =1.00 or higher) and therefore positive for tuberculosis.

**BCG Antigen Specificity of the ELISA kit**

Mouse samples with high concentration of BCG IgGs were also positive with recombinant PDHB (pyruvate dehydrogenase E1 component subunit beta , *M. bovis*) antigen coated plates validating the presence of specific BCG antibodies.

Mice vaccinated with BCG from *M. Bovis*, *M. Tuberculosis*, *M. Butyricum*, *M. Avium* also reacted with BCG antigens isolated *M. Bovis* strain indicating significant crossreactivity among various *Mycobacterium* strains.

BCG antigens are non-specified, complex mixture of many tuberculosis antigens. Mouse antibodies to specific *M. tuberculosis* antigens (MTB 38 kda and MTB 16 Kda) also reacted with BCG antigens used in the kit.

**Antibody isotypes and species crossreactivity**

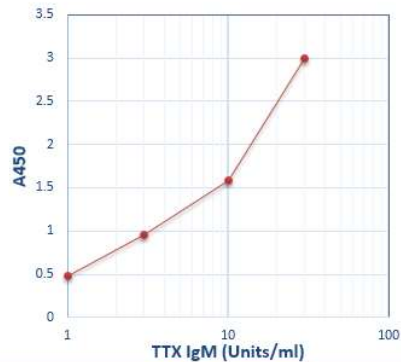
This kit employs anti-IgM-HRP conjugate that reacts with mouse IgM with no significant detection of IgG or IgA. ADI has other kits to detect mouse IgG/IgA against BCG. This kit is designed for mouse samples. ADI has similar kits for other species (rat, G. pig, human, and monkey etc).

**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)	A <sub>450 nm</sub>	Net Mean A <sub>450 nm</sub>	Calculated Conc. (U/ml)
A1, A2	<b>blanks</b>	0.100	-	
B1, B2	<b>Std. A</b> negative control (1 U/ml)	0.58	0.48	
C1, C2	<b>Std. B</b> Cut-off (3 U/ml)	1.06	0.96	
D1, D2	<b>Std. C</b> Weak positive control (10 U/ml)	1.689	1.59	
F1, F2	<b>Std. D</b> positive control (30 U/ml)	3.1	3.0	
G1, G2	<b>Positive control 1</b>	1.40	1.3	

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.



/2\_Nas\_990-220-TMM-ELISA\_Grphs\_

### CALCULATION OF RESULTS

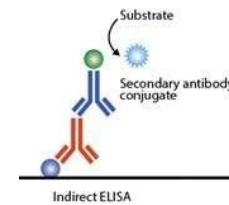
1. Subtract the average blanks A<sub>450</sub> values from the average values of the standards and sample.
2. Plot the NetA<sub>450</sub> values of the standards (y-axis, linear) are plotted against their concentration (x-axis, linear) either on a graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics curves. 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.
3. Results of samples with dilution have to be multiplied with the dilution factor. Samples showing concentrations above the highest standard have to be diluted further and re-tested.

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. 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-BCG (M. Bovis) antibody ELISA kit is based on binding of antibody from serum samples to BCG antigens immobilized on microtiter wells. After a washing step, anti-body-HRP conjugate (subtype specific) is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and blue color developed. The enzymatic reaction (color) is directly proportional to the amount of antibody present in the sample. Adding stopping solution terminates the reaction (converts blue to yellow). Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of antibody in samples is calculated with the supplied standards.

### 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.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site for Prolcin-300 (0.1% v/v in standards, and assay buffers).

[http://4adi.com/commerce/info/showpage.jsp?page\\_id=1060&category\\_id=2430&visit=10](http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10)

## SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for testing. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

**Prepare 1:10 initial stock of all samples** by diluting in 1X sample/conjugate diluent (10 ul sample in 90 ul diluent). The presence of dye does not interfere with the ELISA. Antibodies are stable in this diluent and can be kept at 4oC for weeks. It also avoids freezing and thawing of the original samples. All subsequent test dilutions (1:100 or more) of samples should be made fresh on the day of the test. For example, samples that will be tested at 1:100 will be diluted another 1:10 fold from the 1:10 sample stocks. High antibody samples should be diluted more (e.g. for 1:500 dilution, dilute initial 1:10 stock another 50 fold or 10 ul in 490 ul of 1x diluent).

## REAGENTS PREPARATION

**Dilute Wash buffer** 1:100 with water. Dilute 10 ml stock in 1-L distilled water. Store diluted buffer at 4oC for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes.

**Dilute Sample Diluent** 1:20 with water (1 ml stock in 19 ml water). Keep at 4oC. Prepare as necessary. 1x Sample diluent is used for sample dilution and also for the antibody conjugate (10 ml per plate).

**Dilute antibody-HRP conjugate 1:10** with 1X sample diluent. **Prepare conjugate only in required amounts.** Prepare 1 ml for every strip (100 ul stock in 0.9 ml of 1X sample diluent or prepare 10 ml for full plate). **Do not store diluted conjugate beyond the assay date.**

## 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 (1:100) in 1X sample diluent (see page 2). Standards and 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 RT.
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 **15 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

1. It is a good idea to get familiar with the kit by running just the standards and controls first.
2. Full set of blanks, standards, control must be run with each run.
3. Blanks must not be higher than >0.200. High blanks are indicative of poor washing and high background. In case of high blanks, re-run the standards alone to assure proper functioning of the protocol and reagents.
4. High standards should be >1.00. Low values are indicative of cold reagents, cold temp, short incubation or improper dilution of the conjugate.
5. Positive control A450 value should be >0.500/
6. If any of these criteria is not fulfilled, the results are invalid and the test should be repeated. The assays is calibrated against the ADI reference standards with units assigned arbitrarily. There is no internationally acceptable mouse reference available.