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

| | |
|---|---|
| 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- |
| 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 hemagglutinin (FHA) IgG ELISA kit, 96 |
| 960-310-FMM | Mouse Anti-B. pertussis Filamentous hemagglutinin (FHA) IgM ELISA kit, 96 |
| 960-320-FRG | Rabbit Anti-B. pertussis Filamentous hemagglutinin (FHA) IgG ELISA kit, 96 |
| 960-330-FRM | Rabbit Anti-B. pertussis Filamentous hemagglutinin (FHA) IgM ELISA kit, 96 |
| 960-340-FHG | Human Anti-B. pertussis Filamentous hemagglutinin (FHA) IgG ELISA kit, 96 |
| 960-350-FHM | Human Anti-B. pertussis Filamentous hemagglutinin (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-990-310-TRG

# Rabbit Anti-Tuberculosis (Bacillus Calmette–Guérin/BCG; M. Bovis/M. Tuberculosis) IgG ELISA Kit

## Cat. #990-310-TRG, 96 tests

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

***For Research use only (RUO)***

ALPHA DIAGNOSTIC INTERNATIONAL

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**Rabbit Anti-Tuberculosis (Bacillus Calmette–Guérin/BCG; M. Bovis/M. Tuberculosis) IgG ELISA Kit #990-310-TRG**

| Kit Components | Qty |
|---|---|
| Purified BCG (M. Bovis, vaccine grade) antigens coated microwell strips (96 wells); #990211 | 1 Plate |
| Anti-BCG IgG Standard A (1 U/ml), 1.0 ml ; #990312A | 1 vial |
| Anti-BCG IgG Standard B (3 U/ml), 1.0 ml ; #990312B | 1 vial |
| Anti-BCG IgG Standard C (10 U/ml), 1.0 ml ; #990312C | 1 vial |
| Anti-BCG IgG Standard D (30 U/ml), 1.0 ml ; #990312D | 1 vial |
| Rabbit Anti-BCG IgG Positive Control, 1.0 ml , #990313PC | 1 vial |
| Standards and Controls contain buffer and 0.01% BND as preservative | |
| Anti-Rabbit IgG-HRP Conjugate, 10X, 1.1 ml, #RAB1-1 | 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-990-310 -TRG |

## Intended Use

Rabbit Anti-Tuberculosis (Bacillus Calmette–Guérin/BCG; M. Bovis/M. Tuberculosis) IgG ELISA kit is designed for the detection and measurement of IgG 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 rabbit 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.



## Interpretation of results in rabbits

Naturally occurring, or so-called spontaneous tuberculosis in rabbits is an uncommon finding; most cases are caused by Mycobacterium bovis or M avium. Rabbits apparently become infected when exposed to other tuberculous animals or by ingesting milk from tuberculous cattle. M avium has been reported in rabbits that are housed in close contact with domestic or exotic birds infected with M avium. Rabbits are relatively resistant to M tuberculosis; such infections are seldom reported. Experimental aerosol infection with M. tuberculosis, rabbits develop pulmonary granulomas and occasionally cavities that are histologically similar to those found in humans. Some inbred strains of rabbits are more susceptible to than outbred NZW rabbits to infection with M. tuberculosis.

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 cut-off. All samples higher than the cut-off are positive of BCG IgGs.

## Rabbit Sample Data

Sera from a population of healthy, non-vaccinated young rabbits (NZW, adult, mixed sex; n=8) were tested at 1:100 dilution. All sera showed A450 of less than the Cut-off or <0.300.

## BCG Antigen Specificity of the ELISA kit

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

Rabbit vaccinated with BCG from *M. tuberculosis*, *M. butyricum*, *M. avium* also reacted with BCG antigens isolated from the *M. bovis* strain indicating significant crossreactivity among various Mycobacterium strains.

BCG antigens are non-specified, complex mixture of many tuberculosis antigens. Rabbit 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

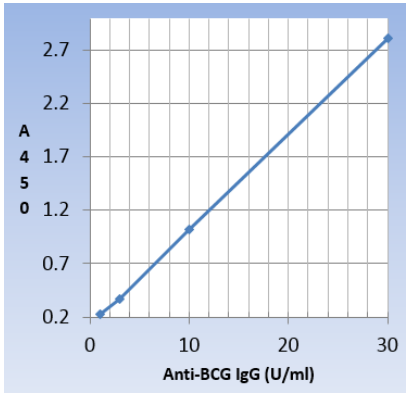
This kit employs anti-IgG-HRP conjugate that reacts with Rabbit IgG with no significant detection of IgM or IgA. ADI has other kits to detect Rabbit IgM/IgA against BCG. This kit is designed for Rabbit samples. ADI has similar kits for other species (rat, G. pig, human, monkey, etc.).

**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) | $A_{450\ nm}$ | Net Mean $A_{450\ nm}$ | Calculated Conc. (U/ml) |
|---|---|---|---|---|
| A1, A2 | **Blank** | 0.100 | - | |
| B1, B2 | **Std. A** negative control (1 U/ml) | 0.329 | 0.229 | |
| C1, C2 | **Std. B** Cut-off (3 U/ml) | 0.47 | 0.37 | |
| D1, D2 | **Std. C** Weak positive control (10 U/ml) | 1.12 | 1.02 | |
| F1, F2 | **Std. D** positive control (30 U/ml) | 2.9 1 | 2.81 | |
| G1, G2 | **Positive control 1** | 1.201 | 1.101 | 9.95 |

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.




### CALCULATION OF RESULTS

1. Subtract the average blanks A450 values from the average values of the standards and sample.
2. The NetA450 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 standard 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.




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 bacillus Calmette–Guérin vaccine (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 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) IgG ELISA kit is based on binding of antibody from serum samples to BCG 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

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices and avoid ingestion or contact of any reagent with skin, eyes, or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid, and BND can be requested or obtained from the ADI website.

## 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). 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 (**1:200** or more) of samples should be made fresh on the day of the test. For example, samples that will be tested at 1:200 will be diluted another 1:20 fold from the 1:10 sample stocks (10 ul of 1:10 stock and 1190 ul of diluent). High antibody samples should be diluted more (e.g. for 1:500-1:5000 dilution).

## REAGENTS PREPARATION

**Wash Solution Concentrate (100x), Cat. #WB-100, 10ml:**

Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at 4ºC for long term and ambient temp. for short term.

**Sample Diluent Concentrate (20x), Cat. #SD-20T, 10ml:**

Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample/Conjugate Diluent** and store at 2-8ºC until the kit lot expires or is used up.

**Anti- Rabbit IgG HRP conjugate (10x), Cat. #RAB1-1, 1.1ml:**

Peroxidase conjugated anti-rabbit IgG in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 100 ul of concentrate to 900 ul of **Working Sample/Conjugate Diluent (1X Sample Diluent)** is sufficient for 1 8-well strip. Use within the working day and discard. Return 10X to 2-8º C storage.

## STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8ºC 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.)*

1. Label and secure the microtiter well strips to be used on the plate. **Dilute** samples (1:100) in 1X sample diluent (see page 3). 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 room temperature (24-28ºC).
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**.
8. Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD reading and data calculations if available. Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

## 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 regents.
4. High standard 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 Rabbit reference available.