

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

Catalog#	ProdDescription
990-530-MTM	Human Anti-S. Typhi (Typhoid) polysaccharide (Vi) IgG ELISA kit, 96 tests,
990-510-HTM	Human Anti-S. Typhi (Typhoid) polysaccharide (Vi) IgM ELISA kit, 96 tests,
990-530-MTM	Mouse Anti-S. Typhi (Typhoid) polysaccharide (Vi) IgG ELISA kit, 96 tests,
990-530-MTM	Mouse Anti-S. Typhi (Typhoid) polysaccharide (Vi) IgM ELISA kit, 96 tests,
990-540-RTG	Rabbit Anti-S. Typhi (Typhoid) polysaccharide (Vi) IgG ELISA kit, 96 tests,
990-550-RTM	Rabbit Anti-S. Typhi (Typhoid) polysaccharide (Vi) IgM ELISA kit, 96 tests,
4200	Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgG ELISA kit
4205	Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgM ELISA kit
4220-AHB	Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) ELISA kit, Quantitative
4300-AHG	Human Anti-Hepatitis A Virus IgG (HAV-IgG) ELISA kit, Quantitative
510-100-HRG	Human Anti-Rubella Virus IgG ELISA kit
510-110-HRM	Human Anti-Rubella Virus IgM ELISA kit
520-100-HMG	Human Anti-Mumps Virus (parotitis) IgG ELISA, 96 tests, Quantitative
520-110-HMM	Human Anti-Mumps Virus (parotitis) IgM ELISA, 96 tests, Quantitative
520-120-HMA	Human Anti-Mumps Virus (parotitis) IgA ELISA, 96 tests, Quantitative
520-200-HVG	Human Anti-Varicella Zoster Virus (chickenpox) IgG ELISA, 96 tests,
520-210-HVM	Human Anti-Varicella Zoster Virus (chickenpox) IgM ELISA, 96 tests, Quantitative
520-220-HVG	Human Anti-Varicella Zoster Virus (chickenpox) IgA ELISA, 96 tests, Quantitative
530-100-HMG	Human Anti-Measles IgG ELISA kit, 96 tests
530-110-HMM	Human Anti-Measles IgM ELISA kit, 96 tests
530-120-HMA	Human Anti-Measles IgA ELISA kit, 96 tests
970-100-PHG	Human Anti-Polio Virus IgG ELISA kits, 96 tests, Quantitative
540-110-DHM	Human Anti-Polio Virus IgM ELISA kits, 96 tests
600-020-HRV	Human Anti-Rabies Virus IgG ELISA Kit, 96 tests, Quantitative
600-120-HRV	Human Anti-Rabies Virus Glycoprotein (RVG) IgG ELISA Kit, 2x 96 tests,
600-220-HRV	Human Anti-Rabies Virus Nucleoprotein (RV-NP) IgG ELISA Kit, 2x 96 tests,
600-300-100	Human Anti-Meningococcal Group A Oligosaccharides-Diphtheria CRM197 IgG
600-300-105	Human Anti-Meningococcal Group CWY Oligosaccharides-Diphtheria CRM197
600-300-115	Human Anti-Meningococcal Group ACWY Oligosaccharides-Diphtheria CRM197
700-140-KLM	Human Anti-KLH IgG (total) ELISA Kit, 2x 96 tests, Quantitative
900-160-83T	Human Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit
910-160-JEM	Human Anti-Japanese encephalitis virus (JEV) IgG specific ELISA kit
910-170-JEM	Human Anti-Japanese encephalitis virus (JEV) IgM specific ELISA kit
920-040-HAG	Human Anti-Influenza A virus IgG ELISA kit
920-050-HAM	Human Anti-Influenza A virus IgM ELISA kit
920-060-HAA	Human Anti-Influenza A virus IgA ELISA kit
920-400-HBG	Human Anti-Influenza B virus Ig's ELISA kit
930-100-TTH	Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-100-DHG	Human Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-110-DHM	Human Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
940-200-DHG	Human Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit
940-210-DHM	Human Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit
950-100-AHA	Human Anti-Adenovirus IgA ELISA kit
950-110-AHG	Human Anti-Adenovirus IgG ELISA kit
950-120-AHM	Human Anti-Adenovirus IgM ELISA kit
960-200-PHA	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA kit,
960-220-PHM	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit,
960-250-PHG	Human Anti-B. pertussis Pertactin IgG ELISA kit
970-100-PHG	Human Anti-Poliomyelitis Virus 1-3 IgG ELISA Kit, 96 tests
980-100-PHG	Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgG ELISA Kit, 96
980-110-PHM	Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgM ELISA Kit, 96
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
AE-320420-1	Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgG ELISA Kit, 96 tests
AE-320430-1	Human Crimean-Congo hemorrhagic fever virus (CCHFV) IgM ELISA Kit, 96 tests
AE-320520-1	Human Anti-Zaire-Ebola virus IgG ELISA Kit, 96 tests

Instruction Manual No. M-990-530-MTM

## Typhoid Vi IgM

### ELISA KIT Cat. # 990-530-MTM

For Determination of Mouse IgM antibodies against *S. enterica* serovar Typhi (Typhoid) polysaccharide (Vi) in Serum or Plasma

*For In Vitro Research Use Only*

**DRAFT - Use manual  
supplied with the kit**



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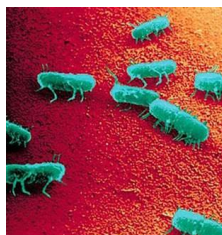
Mouse Anti-S. enterica serovar Typhi (Typhoid) polysaccharide (Vi) IgM ELISA  
# 990-530-MTM (96 tests)

Kit Components (96 tests)	Cat #
S. Typhi Vi antigen coated strip plate, (8x12 or 96 wells) # 990501	1 plate
Typhi Vi IgM Calibrator A (1.8 U/ml) #990532A (1 mL)	1 vial
Typhi Vi IgM Calibrator B (3.6 U/ml) #990532B (1 mL)	1 vial
Typhi Vi IgM Calibrator C (22 U/ml) #990532C (1 mL)	1 vial
Typhi Vi IgM Calibrator D (200 U/ml) #990532D (1 mL)	1 vial
Mouse Typhi Vi IgM positive control #990533PC (1 ml)	1 vial
Anti-Mouse IgM-HRP Conjugate,100X (0.15 ml) # RMCM-2	1 bottle
Sample Diluent, 20X, 10 ml #SD-20TG (green color)	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-990-530-MTM

#### Intended Use

ADI S. enterica serovar Typhi (Typhoid) polysaccharide (Vi) IgM ELISA is intended for the detection and the measurement of S. Typhi Vi-specific IgM antibodies in mouse serum or plasma. This ELISA is particularly designed to assess the antibody levels in mouse vaccinated with new generation S. Typhi Vi based vaccines (Typhim and Typherix). It is intended for research use only (RUO), not for diagnostic procedures.

#### Introduction



Salmonella is a genus of rod-shaped (bacillus) bacteria of the Enterobacteriaceae family. There are two species of Salmonella, **Salmonella bongori** and **Salmonella enterica**. Salmonella enterica is further divided into six subspecies and over 2500 serovars. Salmonellae are found worldwide in both cold-blooded and warm-blooded animals, and in the environment. Strains of Salmonella cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning (Salmonellosis). Salmonella are non-spore-forming, predominantly motile enterobacteria with cell diameters between approximately 0.7 and 1.5 µm, lengths from 2 to 5 µm, and peritrichous flagella (flagella that are all around the cell body). They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes, capable of surviving with or without oxygen.

Typhoid fever or typhoid is a common worldwide bacterial disease transmitted by the ingestion of food or water contaminated with the feces of an infected person, which contain the bacterium Salmonella enterica enterica, serovar Typhi. The disease has received various names, such as gastric fever, abdominal typhus, infantile remittant fever, slow fever, nervous fever and pathogenic fever. The name typhoid means "resembling typhus" and comes from the neuropsychiatric symptoms common to typhoid and typhus. The term

#### Mouse Sample Testing

Eight (8) adult mouse serum samples (Balb/c; mixed sex) samples were analyzed at 1:100 dilution for antibodies to Typhi Vi-IgM using ADI ELISA. All of the sample has raw A45 values in the range of 0.191-0.360 (baseline values).

#### Specificity and Species Cross reactivity

Mouse Anti-S. Typhi Vi IgM ELISA detects mouse IgM with no detection of IgG or IgA. It cannot be used for testing human or rabbit or other species. ADI has separate ELISA kits for mouse, rat, monkey, and other species S. Typhi Vi-IgG or IgM.

#### Other important information

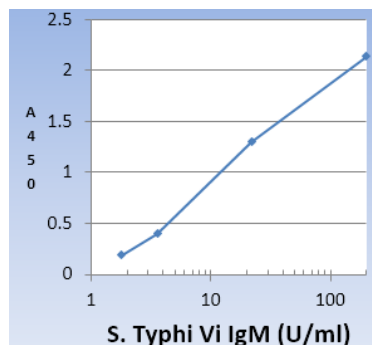
Rabbits immunized with S. Typhi (whole killed bacterium) and two commercially available S. typhi Vi vaccines (purified Vi polysaccharides) and Vi-rEP experimental vaccines were tested in ELISA. Highest titer antibodies were produced by Vi-rEP vaccine but all vaccines produced significant antibody titer after 1 month and 2 month post immunization. Therefore, ADI's Vi-IgG and IgM ELISA may be useful to detect and measure antibodies to Vi-polysaccharides in vaccinated samples.

**References:** Szu SC (2013) Vaccine 31, 1970-1974; FeryL (2004) Clin Exp Immunol;136:297-303;Staats HF (2010) Clin Vaccine Immunol17:412-9 Ivanoff B (1994) Bull WHO 72, 957-971; .Jesudason M (2002) Ind. J. Med. 116, 70-72; Gopalakrishna V (2002) Med J; 43: 354; Olsen SJ (2004) J. Clin. Microbiol. 42, 1885-1889; Shanta D (2006) Infect. Dis. 56, 359-365.

## Calculation of Results

Read A450/630nm values of the wells.

1. Subtract blank (diluent) values from all values including calibrators and samples.
2. Calculated the mean values for Calibrators A-D and samples.
3. Plot the calibrators values (U/ml) Vs the concentration using semi-log scale as shown below. We recommend 4-point curve (do not force linear line).
4. An initial 1:100 dilution has been taken into account. If samples diluted more (e.g. 1:200) then multiply the values by 2 or the fold dilution of the samples.



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Typical Data for demonstration purpose only. Do not use this graphs for calculation. Users must run the calibrators in each kit and calculate values.

## Sensitivity of Vi ELISA

ELISA sensitivity is optimized by antigen coating, antibody conjugate and sample dilution and other assay conditions (incubation time and temperature). Given the optimal ELISA assay testing, this test detects <0.75 u/ml IgG or IgM. Sensitivity is established by sample values that can be clearly distinguished from blank values.

## Quality Control

Absorbance values of the reference control should be similar to the values in the manual. Blanks must be <0.200 as high blanks >0.3 indicate poor washing or too much conjugate. In case of high blanks, increase number of washings.

High positive calibrator must be >1.000.

The **positive control** must be A450=0.500-1.00.

Repeated significant divergence of calibrators and controls indicates technical errors or deterioration or expiration of kit components.

enteric fever is a collective term that refers to severe typhoid and paratyphoid. The bacterium that causes typhoid fever may be spread through poor hygiene habits and public sanitation conditions, and sometimes also by flying insects feeding on feces. An estimated 16–33 million cases of typhoid fever occur annually. Its incidence is highest in children and young adults between 5 and 19 years old. These cases as of 2010 caused about 190,000 deaths up from 137,000 in 1990.

S. Typhi expresses a number of immunogenic structures on the surface, some of which provide a basis for serology identification. These include O (lipopolysaccharide), H (flagella) and the somewhat less immunogenic Vi capsule. S. Typhi exhibiting variation in these antigens are uncommon, with notable exceptions. S. Typhi found in Indonesia express variant H antigens including H:j and H:z66. Vi-negative S. Typhi isolates have been reported in Pakistan but are rare. Therefore, S. Typhi expressing O (O9, O12), Vi and H:d are ubiquitous in most endemic areas. Seroprevalence studies have been performed in endemic regions to determine antibody titers to O, H and Vi in the general population. Many individuals in endemic areas have cross-reactive antibodies even though they have no clinical record of typhoid. Additionally, such raised antibody levels frequently cannot be detected in patients with culture confirmed typhoid. Problems have also been encountered during the testing of commercial serological tests, including Typhidot and Tubex.

Diagnosis is made by any blood, bone marrow or stool cultures and with the Widal test (demonstration of salmonella antibodies against antigens O-somatic and H-flagellar). The **Widal test** is time-consuming, and often, when a diagnosis is reached, it is too late to start an antibiotic regimen. Typhidot-M is a dot enzyme immunoassay for the detection of specific IgG/IgM antibody to Salmonella typhi OMP antigen Salmonella typhi.

There are few vaccines licensed for use for the prevention of typhoid: **the live, oral Ty21a vaccine** (sold as Vivotif by Crucell) and the injectable **Typhoid polysaccharide vaccine** (sold as Typhim Vi by Sanofi Pasteur and Typherix by GlaxoSmithKline). Both are 50% to 80% protective and are recommended for travellers to areas where typhoid is endemic. There exists an **older, killed-whole-cell vaccine** that is still used in countries where the newer preparations are not available, but this vaccine is no longer recommended for use because it has a higher rate of side effects (mainly pain and inflammation at the site of the injection). A new vaccine based upon Vi-rEPA (recombinant Pseudomonas aeruginosa exoprotein A) has been shown to confer 90% protection for 4 years in 2-5 yrs old children.

## PRINCIPLE OF THE TEST

Alpha Diagnostic's Typhoid Vi IgG/IgM antibody test kit is based on the principle of indirect ELISA). Vi antigen is bound on the surface of the microtiter strips. Diluted sample or standards are pipetted into the wells of the microtiter plate. Vi IgG/IgM antibodies bind to the immobilized Vi antigen. After incubation at room temperature, the plate is rinsed with wash buffer and plates are incubated with anti-human-IgG peroxidase conjugate. After another washing step, the substrate (TMB) solution is added that produces blue color in the antibody positive wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The color is measured using an ELISA reader at 450 nm. The concentration of the IgG/IgM antibodies is directly proportional to the intensity of the color. Results can also be expressed as -ve or +ve in comparison to the cut-off standards.

## MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Distilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm). 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 Proclin-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](http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10)

## PRECAUTIONS

Do not ingest or swallow and reagents. No reagents from different kit lots have to be used, they should not be mixed among one another. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

## 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 40C 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).

Users may choose to test their samples at a dilution different than 1:100 recommended in this manual (e.g. 1:50 or 1:200 etc) and compare the control groups with experimental group or vaccinated vs non-vaccinated. A difference in the A450 values of one group Vs another should be indicated of the Vi antibody.

## REAGENTS PREPARATION

**Dilute Wash buffer** 1:100 with water. Dilute 10 ml stock in 1-L distilled water. Store diluted buffer at 40C 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 40C. 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 1:100** with 1X sample diluent. **Prepare conjugate only in required amounts.** Prepare 1 ml for every strip (10 ul stock in 1 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 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.

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

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 40C. Do not touch the bottom of the wells.

### ELISA Test Procedure

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate. Prepare 1x Wash buffer, 1x sample diluent, and 1x antibody conjugate. **All samples should be diluted 1:100 (see sample preparation). Do not dilute the calibrators.** We recommend preparing a replica plate samples on ELISA tubes or blank ELISA plates for a quick transfer of samples to the coated plate using multi-pipette.

1. Dispense **100 ul of sample** diluent (blanks; Wells A1/A2), 100 ul of pre-diluted supplied calibrators (wells B1/B2-D1/D2, Positive control (E1/E2), and diluted samples (F1/F2 etc) in duplicate. Gently mix the wells by tapping against the palm for 5-10 seconds. Cover the plate with aluminum file and **incubate the plate at RT (25-28oC) for 60 mins.**
2. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 300 ul of 1X 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.
3. Add **100 ul of diluted anti-IgM-HRP conjugate** to all wells leaving one empty for the substrate blank. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp (25-28oC).
4. Wash the wells 3 times as in step 3.
5. Add **100 ul TMB substrate solution.** This time also the substrate blank is pipetted. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temp. Blue color develops in positive controls and samples.
6. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).
7. Measure the **absorbance at 450 nm** and a reference filter 630nm using an ELISA reader within 15 min.

### WORKSHEET OF A TYPICAL ASSAY

Wells	Calibrators /Samples	A450	Net A450	Results
A1/A2	Sample diluent	0.1000	-	
B1/B2	Calibrator A (1.8 U/ml)	0.38	0.28	
C1/C2	Calibrator B (3.6 U/ml)	0.54	0.44	
D1/D2	Calibrator C (22 U/ml)	1.5	1.40	
D1/D2	Calibrator D (200 U/ml)	2.54	2.44	
E1/e2	Positive control	1.15	1.05	+ve
F1/F2	Sample 1	0.410	0.310	-ve
G1/G2	Sample 2	0.910	0.900	+ve