Wantai Mycobacterium Tuberculosis Diagnostics

WANTAI TB-IGRA

Diagnostic Kit for T Cell Infected with Mycobacterium Tuberculosis (TB-IGRA)

REF **WT-1196** | **V. 2018-01 [Eng.]** | Σ **28** | IVD

Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of WANTAI TB-IGRA achieved.

INTENDED USE

WANTAI TB-IGRA is an enzyme-linked immunosorbent assay for quantitative detection of Interferon Gamma (IFN-γ) that responds to in-vitro stimulation by Mycobacterium tuberculosis antigens in human whole blood. It is intended for use as an aid in the diagnosis of tuberculosis (TB) infection.

SUMMARY

Tuberculosis (TB) is one of the most important health threatening problems worldwide. The World Health Organization estimates that one third of the world's population is infected with M. tuberculosis. Each person carrying latent TB infection (LTBI) has approximately a 10% chance of progressing to active disease. This rate of progression is elevated among certain groups, including those who have been recently infected and those with a weakened immune system.

IGRA measure a person's immune reactivity to M. tuberculosis. T lymphocytes from most persons that have been infected with M. tuberculosis will release interferon gamma (IFN-γ) when mixed with antigens (substances that can produce an immune response) derived from M. tuberculosis. Interferon Gamma Release Assay (IGRA) is whole-blood test that can aid in diagnosing Mycobacterium tuberculosis infection, including both latent tuberculosis infection (LTBI) and tuberculosis (TB) disease.

PRINCIPLE OF THE TEST

This kit uses the principle of IGRA combined with enzyme linked immunosorbent assay (ELISA) to measure specific antigen mediated immune response strength. The antigens selected for stimulation is specific fragments that pathogenic Mycobacterium tuberculosis have, but BCG vaccine and other Mycobacterium do not have. The patients infected with Mycobacterium tuberculosis have specific T lymphocytes which can identify these antigens and T lymphocytes will be stimulated to proliferate and release cytokines such as IFN-γ. This kit uses ELISA method for quantitative detection the specific IFN-γ released by cells to determine Mycobacterium tuberculosis infection.

Polystyrene microwell strips are pre-coated with mouse anti-human IFN-γ IgG monoclonal antibody. During the first incubation step, the IFN-γ, if present, will be bound to the solid phase pre-coated anti-IFN-γ antibody. The wells are washed to remove unbound proteins, and anti-IFN-γ antibody conjugated to horseradish peroxidase (HRP) are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any complex previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing Tetramethyl benzidine (TMB) and urea peroxide are added to the wells. In presence of immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. Wells containing no IFN-γ remain colorless.

The amount of color intensity can be measured and is proportional to the amount of IFN-γ captured in the wells, and to the specimen respectively. The concentration of IFN-γ can be calculated by standard concentration and absorbance value (A value).

COMPONENTS

IVD In Vitro Diagnostic Use Only

This kit contains reagents sufficient for testing of maximum of 28 specimens in a test run.

UUU | PLATE
Code 5 (1x96wells)
8×12/12×8-well per plate

MICROWELL PLATE: Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains mouse anti-human IFN-γ IgG monoclonal antibody. The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once opened, stable for 4 weeks at 2-8°C.

TUBE | N
Code N (28x30μl per vial)
preserv.0.1% ProClin™ 300

BACKGROUND CONTROL CULTURE TUBE (N): Colorless liquid filled in blue color vial.
Culture medium.
Ready to use as supplied.

TUBE | T
Code T (28x30μl per vial)
preserv.0.1% ProClin™ 300

TESTING CULTURE TUBE (T): Colorless liquid filled in white color vial.
TB specific stimulating antigen in culture medium.
Ready to use as supplied.

TUBE | P
Code P (28x30μl per vial)
preserv.0.1% ProClin™ 300

POSITIVE CONTROL CULTURE TUBE (P): Colorless liquid filled in pink color vial.
TB non-specific stimulating antigen in culture medium.
Ready to use as supplied.

STANDARD
Code S (2x2mg per ampoule)
preserv.0.1% ProClin™ 300

STANDARD: Freeze-dried Standard filled in ampoule.
IFN-γ positive substance.
Should be reconstituted to a concentration of 400pg/ml. Once prepared, use on the same day only.

HRP | CON
Code 6 (1x6ml per vial)
preserv.0.1% ProClin™ 300

HRP-CONJUGATE: Red liquid filled in a white vial with red screw cap.
Horseradish peroxidase-conjugated mouse anti-human IFN-γ IgG monoclonal antibody.
Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

DIL | SPE
Code 9 (1x3ml per vial)
preserv.0.1% ProClin™ 300

SPECIMEN DILUENT: Yellowish liquid filled in a white vial with blue screw cap.
Protein-stabilized buffer, BSA, and sucrose solution.
Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

DIL | STD
Code 11 (1x8ml per vial)
preserv.0.1% ProClin™ 300

STANDARD DILUENT: Yellowish liquid filled in a white vial with brown screw cap.
Protein-stabilized buffer, casein, and sucrose solution.
Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

WASH | BUF | 20X
Code 1 (1x50ml per bottle)
DILUTE BEFORE USE!
detergent Tween-20

WASH BUFFER: Colorless liquid filled in a clear bottle with white screw cap.
Buffer solution containing surfactant.
The concentrate must be diluted **1 to 20** with distilled/ deionized water before use. Once diluted, stable for 1 week at room temperature, or for 2 weeks when stored at 2-8°C.

CHROM | SOL | A
Code 2 (1x6ml per vial)

CHROMOGEN SOLUTION A: Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution.
Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

CHROM | SOL | B
Code 3 (1x6ml per vial)

CHROMOGEN SOLUTION B: Colorless liquid filled in a black vial with black screw cap. TMB (Tetramethyl benzidine), N,N- dimethylformamide.
Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

STOP | SOL
Code 4 (1x6ml per vial)

STOP SOLUTION: Colorless liquid in a white vial with yellow screw cap.
Diluted sulfuric acid solution (0.5M H_2SO_4).
Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

- **PLASTIC SEALABLE BAG:** For enclosing the strips not in use — 1 unit
- **PACKAGE INSERT** — 1 copy
- **CARDBOARD PLATE COVER** — 3 sheets

To cover the plates during incubation and prevent evaporation or contamination of the wells.

MATERIALS REQUIRED BUT NOT PROVIDED

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, dry incubator or water bath, 37±1°C, plate reader, single wavelength 450nm or dual wavelength 450/600~650nm, microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. Fresh whole blood specimens are required for this assay. Whole blood specimens must be collected in **endotoxin-free** blood collection tubes with **lithium heparin as anticoagulant (BD Vacutainer or Greiner Bio-One lithium heparin blood collection tube is highly recommended)**.
2. Hemolyzed specimens or specimens containing suspended fibrin or aggregates must not be used as they could give erroneous results in the assay. Icteric specimens (bilirubin content is not higher than 1.71mmol/l) and lipemic specimens (triglyceride content is not higher than 170mmol/l) can be used.
3. The whole blood specimens should be respectively dispensed into "N", "T" and "P" culture tubes in turn within 16 hours after collection, and then place the culture tubes in incubator at 37°C to culture.
4. Do not ice-bath or freeze specimens, this can cause cell death.
5. After culture, the plasma is separated from the whole blood specimens by centrifugation. The plasma separated can be stored at 2-8°C for 2 days. For long-term storage, should be frozen at -20°C or lower, multiple freeze-thaw cycles should be avoided.
6. For shipment, specimens should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical specimens and ethological agents.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of WANTAI TB-IGRA, during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

TO BE USED ONLY BY QUALIFIED PROFESSIONALS

The ELISA assays are time and temperature sensitive. To avoid incorrect result, ***strictly follow the test procedure steps and do not modify them***.

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. **CAUTION - CRITICAL STEP**: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.
4. Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
5. Do not touch the exterior bottom of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid long time interruptions of assay steps. Assure same working conditions for all wells.
8. Calibrate the pipette frequently to assure the accuracy of specimens/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
9. Assure that the incubation temperature is 37°C inside the incubator.
10. When adding specimens, do not touch the well's bottom with the pipette tip.
11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/600~650nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.
14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
15. **WARNING:** Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for HBsAg and antibodies to HIV 1/2, HCV, TP. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
17. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
18. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.
19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
20. The Stop solution 0.5M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
21. ProClin™ 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

INDICATIONS OF INSTABILITY DETERIORATION OF THE REAGENT: Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the specimens must be retested. In case of constant erroneous results and proven deterioration or instability of the reagents, immediately substitute the reagents with new one or contact Wantai technical support for further assistance.

Warning:
H317, P280, P333+P313, P363
ProClin™ 300

Danger:
H360D, P201, P280, P308+P313
N,N- dimethylformamide

PROCEDURE

1) In Vitro Release of IFN-γ

Step 1 **Specimen Collection:** Collect whole blood specimens from patients by venipuncture. The volume collected should be not less than 4ml. Whole blood specimens must be collected in blood collection tubes with lithium heparin as anticoagulant (BD Vacutainer or Greiner Bio-One lithium heparin blood collection tube is highly recommended), well mixed with anticoagulant and stored at 20-27°C for maximum 16 hours before the next dispensing step.

Step 2 **Specimen Dispensing:** Gently shake the collection tubes upside down at least 3-5 times to mix the whole blood specimens before dispensing. The whole blood specimens should be respectively dispensed into "N", "T" and "P" culture tubes in turn within 16 hours after collection. Dispense 1ml of whole blood specimen for each culture tube.

Step 3 **Culture:** Gently shake the culture tubes upside down 5 times and then immediately place the culture tubes in incubator at 37°C to culture for 22±2 hours. The tubes should be kept upright during the culture.

Step 4 **Centrifugation:** After the culture, centrifuge the whole blood specimens at 3000-5000rpm for 10 minutes to separate plasma and red blood cells, and then take the plasma for the following procedures of ELISA. Ensure that only the plasma is taken from the whole blood without red blood cells.

2) Quantitative Determination of IFN-γ

Step 1 **Reagents preparation:** Allow the reagents and specimens to reach room temperature (18-30°C) for at least 15-30 minutes. Check the Wash Buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the 20X concentrated Wash Buffer 1 to 20 with distilled or deionized water. Use only clean vessels to dilute the buffer.

Step 2 **Standards preparation:** Add distilled or deionized water into ampoule according to the volume indicated on the label of ampoule to reconstitute the freeze-dried standard. After 2-3 minutes, the standard is completely dissolved, gently mix until it is homogeneous, then 400pg/ml standard is ready to use. Use doubling dilution method to dilute the above standard with Standard Diluent to 200pg/ml, 100pg/ml, 50pg/ml, 25pg/ml and 12.5pg/ml. Then the final concentrations of the ready-to-use Standards are 400pg/ml, 200pg/ml, 100pg/ml, 50pg/ml, 25pg/ml and 12.5pg/ml respectively.

Step 3 **Numbering Wells:** Set the strips needed in strip-holder and number sufficient number of wells including one well for the plasma specimen of each culture tube, two wells for each standard and one well for Blank (neither specimens nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test. The standards should be assayed in duplicate.

Step 4 **Adding Specimen Diluent:** Add **20μl** of Specimen Diluent into each well except the Blank well.

Step 5 **Adding Specimen:** Add **50μl** of the Standards and **50μl** of specimen into their respective wells except the Blank well and mix by tapping the plate gently. **Note:** Use a separate disposal pipette tip for each specimen as to avoid cross-contamination.

Step 6 **Incubating 1:** Cover the plate with the plate cover and incubate **at 37°C for 60 minutes**. It is recommended to use thermostat-controlled water tank as to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step 7 **Adding HRP-Conjugate:** Add **50μl** of HRP-Conjugate Reagent into each well except the Blank well and mix by tapping the plate gently. **Note:** Never add HRP-Conjugate to the Blank well; Add HRP-Conjugate directly without washing step after above incubation step.

Step 8 **Incubating 2:** Cover the plate with the plate cover and incubate **at 37°C for 60 minutes** again.

Step 9 **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time, allow the microwells to soak **for 30-60 seconds**. After the final washing cycle, turn the plate onto blotting paper or clean towel, and tap it to remove any remainders.

Step 10 **Coloring:** Add **50μl** of Chromogen Solution A and then **50μl** of Chromogen Solution B into each well including the Blank well and mix by tapping the plate gently. Incubate the plate **at 37°C for 15 minutes avoiding light**. The enzymatic reaction between the Chromogen Solution A/B and the HRP-Conjugate produces blue color in the Standards wells and in IFN-γ positive specimen wells.

Step 11 **Stopping Reaction:** Using a multichannel pipette or manually add **50μl** of Stop Solution into each well and mix gently. The blue color will turn yellow after stopping the reaction.

Step 12 **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance value (A value) **at 450nm**. If a dual filter instrument is used, set the reference wavelength **at 600~650nm**. Calculate the results (**Note:** read the absorbance value **within 10 minutes** after stopping the reaction).

INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential in order to obtain correct and precise analytical data.
2. It is therefore, recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than **5 automatic washing cycles** of **350-400μl/well** are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out **5 washing cycles,** dispensing **350-400μl/well** and aspirating the liquid for **5 times**. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before they are wasted in an appropriate way.
7. The concentrated Wash buffer should be diluted **1:20** before use. If less than a whole plate is used, prepare the proportional volume of solution.

INTERPRETATION OF RESULTS AND QUALITY CONTROL

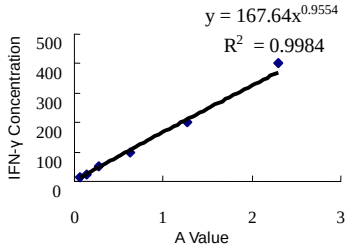
If the result reading is based on single filter plate reader, the results should be calculated by subtracting A value of the Blank well from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract the A value of Blank well from the print report values of specimens and controls.

The limit of detection of this kit is 2pg/ml, the detection range is 2pg/ml~400pg/ml. The effective linear range of the kit is 12.5pg/ml~400pg/ml, if the concentration of IFN-γ in specimen is higher than 400pg/ml, it is necessary to redo the test after diluting the specimen by using Standard Diluent.

1. Use the antigen concentrations of the Standards (12.5pg/ml~400pg/ml) and the mean value of its corresponding absorbance values to do power curve fitting (standard curve) so as to determine the linear regression equation, then substitute the absorbance values of plasma specimens cultured in N, T and P culture tubes into the above equation to obtain the corresponding IFN-γ concentration.

   An example is as follows:
   the mean of absorbance value of Standards is as the independent variable ($\overline{X}$), its corresponding IFN-γ concentrations (12.5pg/ml~400pg/ml) is as the dependent variable (Y), and then do the power fitting, thus the linear regression equation determined is $y=167.64x^{0.9554}$, the data and the graph are as follows:

Mean of A Values of Standard	2.294	1.277	0.621	0.275	0.136	0.065
IFN-γ Concentration of Standard (pg/ml)	400	200	100	50	25	12.5



   If the A value of T culture tube of one specimen is A=1.526, substitute it into the equation, the IFN-γ concentration of T culture tube calculated is: 167.64*Power (1.526, 0.9554) = 251 (pg/ml). (Note: The standard curve is for illustration only.)

2. Interpretations of results:
   The concentration of Testing Culture Tube (T) = T, the concentration of Background Control Culture Tube (N) = N, The concentration of Positive Control Culture Tube (P) = P (Unit: pg/ml)

N	P-N	T-N	Result	Interpretation
≤400	any value	≥14 and ≥ $\frac{N}{4}$	positive	Infected with Mycobacterium tuberculosis (active, latent or inapparent infection)
	≥20	<14	negative	Not infected with Mycobacterium tuberculosis
	≥20	≥14 but < $\frac{N}{4}$	negative	
	<20	<14	indeterminate	Cannot determine whether Mycobacterium tuberculosis infection
	<20	≥14 but< $\frac{N}{4}$	indeterminate	
>400	any value	any value	indeterminate	

3. Quality Control:
   If any following result is obtained, the test results should be considered invalid, it is necessary to repeat the test: (1) The correlation coefficient of dose-response curve (r)<0.9900; (2) The mean of A values of 400pg/ml Standard <1.0.

PERFORMANCE CHARACTERISTICS

1. Total 1228 specimens were tested in parallel with this kit and QuantiFERON-TB Gold (QFT) as a reference kit, the positive coincidence rate between two kits is 94%, the negative coincidence rate is 83%, the total coincidence rate is 89%, Kappa value is 0.77.

	Sensitivity (n=875)	Specificity (n=353)	Accuracy
Wantai	78.3%	76.2%	77.7%
QFT	74.6%	79.3%	76.0%

2. The specimens from different classifications of TB patients were tested with this kit, QFT and bacteriological test respectively, the results are as follows:

Specimen classification	Number	Bacteriological positive*	Wantai (+)	QFT (+)
Pulmonary tuberculosis	721	444	591	564
A single extrapulmonary tuberculosis	35	5	28	29
Tuberculosis with other diseases	30	9	18	17
Total	786	458	637	610
Sensitivity		58.3%	81.0%	77.6%

*Bacteriological positive is one or both positive of bacteria culture and smear testing.

3. The endogenous proteins, e.g. tumor necrosis factor-a (TNF-α), interferon α-2a (IFNα-2a), interferon α-2b (IFNα-2b), ω interferon (IFN-ω), mouse interferon-γ (mIFN-γ), interleukin-2 (IL-2), Interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-18 (IL-18), interleukin-21 (IL-21), interleukin-32 (IL-32), under the concentration of 40ng/ml do not have influence on this kit; Specimens positive for rheumatoid factor, antinuclear antibodies, and Sjogren's syndrome patients, lupus erythematosus patients do not have influence on this kit.

4. 265 specimens from BCG vaccination groups (121 cases with active infection, 91 cases with latent infection and 53 cases of healthy people) were tested with this kit and QFT. This kit detected 133 positive, 132 negative. The QFT detected 115 positive, 150 negative. The positive coincidence rate of the two kits is 100%, the negative coincidence rate is 88%, and the total coincidence rate is 93%.

LIMITATIONS

1. Results from this kit must be used in conjunction with each individual's epidemiological history, current medical status, and other diagnostic evaluations.
2. TB-IGRA negative results cannot confirm no M. tuberculosis infection. Some TB-IGRA negative may be caused by: improper handling of specimens, such as severe operating result in cell damage; Immune deficiency of subjects, such as immunosuppressive therapy or AIDS; or other possible causes.
3. In theory, every subject can produce an immune response against non-specific stimulating antigen PHA.
4. TB-IGRA positive results may also be caused by rare mycobacterium (M. kansasii, M. szulgai, M. marinum).
5. Specimens collected have to be fresh whole blood with only lithium heparin as anticoagulant. Some blood collection tubes contain a high level of endotoxin, which may cause false positive. Therefore, it is highly recommended to use BD Vacutainer or Greiner Bio-One lithium heparin blood collection tube.
6. With the increase of PPD strength, the positive rate of this kit could be from low to high, showed a significantly higher trend, the strength of PPD is positively correlated with the positive rate of this kit.
7. Common sources for mistakes are: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, equipment, timing, volumes, specimen nature and quality.
8. Avoid carrying out the assay under an environment with volatile substance and hypochlorite disinfectant (e.g. javel water). The whole blood specimens collected should be transferred into culture tubes for culture as soon as possible within 16 hours after collection in order to prevent cell sedimentation. If placed more than 1 minute after the collection, should mix gently before transferring to culture tubes. If placed more than 16 hours, it is invalid for the assay.
9. Before dispensing whole blood specimens, gently shake the collection tubes upside down to mix. The whole blood specimens should be respectively dispensed into "N", "T" and "P" culture tubes in turn within 16 hours after collection. The tubes should be kept upright during the culture.
10. Culture tubes should be centrifuged at 3000-5000rpm for 1 minute before use in order to concentrate the culture solution in the bottom of culture tubes. If any turbidity observed in culture tubes before use, it cannot be used for the assay.
11. After the blood culture, must centrifuge culture tubes before taking plasma, otherwise it will lead to the increase of background.
12. The Standard is freeze-dried, it must be completely dissolved before use. It is recommended to use doubling dilution to dilute the standard from high concentration to low concentration. The reconstituted standards should only be used within the same day, cannot be frozen for storage.
13. At the coloring step of the assay, must dispense Chromogen Solution A first, and then dispense Chromogen Solution B in order to avoid low coloring.
14. Each assay must be performed with the standards, test results must be calculated by current standard curve, otherwise it may result in large errors for quantitative results.
15. If the value of only one out of six prepared standards is significantly higher or lower, and it is caused by human error, then can give up this point and plot the standard curve with the other standards.
16. The prevalence of the marker will affect the assay's predictive values.
17. If, after retesting of the initially reactive specimens, the assay results are negative, these specimens should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step. For more information regarding Wantai Troubleshooting, please refer to Wantai's "Troubleshooting Guide", or contact Beijing Wantai technical support for further assistance.
18. This kit is intended ONLY for testing of individual whole blood specimens. Do not use it for testing of cadaver specimens, saliva, urine or other body fluids, or pooled (mixed) blood.

REFERENCES

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4. Doherty, T.M., et al. Immune responses to the Mycobacterium tuberculosis-specific antigen ESAT-6 signal subclinical infection among contacts of tuberculosis patients. J Clin Microbiol, 2002. 40: 704-6.
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SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:

Use this summary only as a reference and always follow the comprehensive method sheet when performing the assay.
Note: the components of individual kits are not lot- interchangeable.

1. Microwell plate	Code 5	one
2. Background Control Culture Tube	Code N	28x30μl
3. Testing Culture Tube	Code T	28x30μl
4. Positive Control Culture Tube	Code P	28x30μl
5. Standard	Code S	2 ampoules
6. HRP-Conjugate	Code 6	1x6ml
7. Specimen Diluent	Code 9	1x3ml
8. Standard Diluent	Code 11	1x8ml
9. Wash Buffer	Code 1	1x50ml
10. Chromogen Solution A	Code 2	1x6ml
11. Chromogen Solution B	Code 3	1x6ml
12. Stop Solution	Code 4	1x6ml

SUMMARY OF THE ASSAY PROCEDURE:

Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

Add Specimen Diluent	20μl
Add Specimen	50μl
Incubate	60 minutes
Add HPR-Conjugate	50μl
Incubate	60 minutes
Wash	5 times
Coloring	50μl A + 50μl B
Incubate	15 minutes
Stop the reaction	50μl stop solution
Read the absorbance	450nm or 450/600~650nm

EXAMPLE SCHEME OF CONTROLS / SPECIMENS DISPENSING:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	STD4	S2(N)									
B	STD1	STD5	S2(T)									
C	STD1	STD5	S2(P)									
D	STD2	STD6	…									
E	STD2	STD6	…									
F	STD3	S1(N)	…									
G	STD3	S1(T)										
H	STD4	S1(P)										

CE MARKING SYMBOLS:

IVD **In Vitro Diagnostic Medical Device**

+2°C~+8°C Storage Conditions

Use By

LOT **Batch**

Content Sufficient For <n> Tests

Instructions For Use

CE **CE Marking – IVDD 98/79/EC**

EC REP **EU Authorized Representative**

REF **Catalog Number**

Manufacturer

Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.
No.31 Kexueyuan Road, Changping District, Beijing 102206, China
Tel: +86-10-59528888, Fax: +86-10-89705849
Website: www.ystwt.com
Email: wtexport@ystwt.com

EC REP **Qarad b.v.b.a.**
Cipalstraat 3, B-2440 Geel, Belgium
Email: qarad@qarad.com

CE

Version: V. 2018-01 [Eng.]
Issuing Date: May 25, 2018
Number of revision: Revision 3