LIOFeron® TB/LTBI

HUMAN IFN-γ ELISA Instructions for Use

22 Tests — **REF LIO-Feron 02_22**

44 Tests — **REF LIO-Feron 02_44**

Interferon Gamma Release Assay for the detection of an infection with *Mycobacterium tuberculosis*.

IVD In vitro diagnostic medical device

CE

LIONEX GmbH
Salzdahlumer Straße 196, Geb. 1A
38126 Braunschweig, GERMANY

www.lionex.de
sales@lionex.de

Revision EN_A / 250902

Document Revision History

Date	Revision	Changes
2025-09-02	EN_A	General revision for adaptation to the regulation (EU) 2017/746 on in vitro diagnostic medical devices

For more information and technical support please contact:

LIONEX GmbH

Salzdahlumer Straße 196, Gebäude 1A
38126 Braunschweig, GERMANY
Telephone: +49 (0) 531 - 260 12 66
Fax: +49 (0) 531 - 618 06 54
E-Mail: sales@lionex.de
Web: www.lionex.de

Contents

Intended Use

LIOFeron® TB/LTBI is an in vitro diagnostic medical device for the quantitative determination of Interferon gamma (IFN-γ) in human blood after stimulation with specific antigens of *Mycobacterium tuberculosis*.

The test is suitable for the diagnosis of latent tuberculosis infection (LTBI) but cannot distinguish between active and latent tuberculosis. According to the WHO consolidated guidelines on tuberculosis (version 2022), a positive result by itself is not sufficient for a definitive diagnosis and should be confirmed by other tests such as organ-related imaging, bacteriological and microbiological procedures.

LIOFeron® TB/LTBI is a 2-component kit, consisting of the components **HUMAN BLOOD STIMULATION TUBES** and **HUMAN IFN-γ ELISA**.

The test is for professional use only. The device is NOT for self-testing.

Information on Tuberculosis

Human tuberculosis has developed into a global disease with its resurgence in Western countries in recent decades. TB is predominantly a disease of the respiratory tract but can also affect other organs. People with active TB are highly infectious. Transmission of active TB most commonly occurs by droplet infection when coughing or sneezing via inhalation through mouth or nose. Latent TB infection (LTBI) is not contagious and occurs when the immune system has been able to suppress a primary TB infection. LTBI remains in the body for life and can break out when the immune system is weakened, such as in old age, cancer or due to infection with other diseases.

According to estimates by the World Health Organisation (WHO), TB is one of the leading causes of death worldwide and is likely to top the list of infection related causes of death again, as it did before the COVID-19 pandemic. In 2023 TB caused around 1.25 million deaths (variation from 1.13-1.37 million), including 1.09 million deaths in HIV-negative patients and an additional 161,000 deaths in HIV-positive patients. According to estimates, there are more than 10.0 million new TB cases per year (variation from 10.1-11.7 million) with an upward trend in recent years[24].

Drug resistance in HIV treatment and the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of the tuberculosis pathogen *M. tuberculosis* are making treatment increasingly difficult, leading to hopeless situations. WHO estimates, 400,000 MDR-TB cases were detected and reported worldwide in 2023[24].

In addition, there is no effective vaccination against HIV and TB, which further aggravates the situation. *M. bovis* BCG, the only available vaccine against TB, has proven to be ineffective and the vaccine is often inactive[25].

Application Field

The diagnosis of an infection with TB can be helpful in deciding how to organise therapy. Concentrations of various cytokines in human blood have proven to be a significant parameter for the diagnosis of infections, including infections with *M. tuberculosis*.

T lymphocytes from patients infected with *M. tuberculosis* recognise mycobacterial antigens and trigger cytokine production, e.g. IFN-γ [9, 12-14]. The **LIOFeron® TB/LTBI** interferon-gamma release assay (IGRA) is a qualitative test based on the quantitative determination of IFN-γ and can be helpful for the diagnosis of active and latent TB. However, IGRAs cannot distinguish between active and latent TB.

IGRAs are widely used to measure IFN-γ production by human peripheral blood mononuclear cell (PBMCs) in active and latent TB after exposure to recombinant antigens from *M. tuberculosis*. The WHO has endorsed IGRAs as the preferred test for the detection of latent TB infection (LTBI) in BCG-vaccinated individuals in low TB burden countries.

Other fields of application can be found in the most recent version of the WHO consolidated guidelines on tuberculosis.

Test Principle

The LIOFeron® TB/LTBI consists of two main components, the HUMAN BLOOD STIMULATION TUBES and the HUMAN IFN-γ ELISA. The test is a cytokine detection test based on the fact, that human blood cells produce IFN-γ when exposed to specific *M. tuberculosis* antigens.

LIOFeron® TB/LTBI contains two different TB antigen tubes, TB A and TB B. TB A contains the antigens ESAT6, CFP10 and TB 7.7, which are commonly used for IGRA tests[2-4, 7, 10, 11, 17, 19, 20]. TB B contains a patented antigen from LIONEX GmbH with CD8+ epitopes[4, 18].

Per Test the HUMAN BLOOD STIMULATION TUBES contain one positive control tube, one negative control tube und two TB antigen tubes. The human blood sample is collected from a vein into a blood collection tube containing lithium heparin (not included in the kit). Then 1 mL each of the sample is pipetted into the negative control, positive control and TB antigen tubes of the kit.

The samples in the tubes are carefully mixed by inverting the tubes and left in an incubator at 37 °C overnight. The next day, the clear supernatant (plasma) is carefully separated from the pellet (blood cells, red) and analysed using the HUMAN IFN-γ ELISA. The ELISA is used to determine the concentration of IFN-γ produced after the blood cells come into contact with the specific *M. tuberculosis* antigens. These specific antigens differ from the antigens found in BCG and in most other mycobacteria, that do not belong to the *M. tuberculosis* complex (NTM).

The HUMAN IFN-γ ELISA is based on the principle of the enzyme immunoassay (EIA). Specific monoclonal anti-human IFN-γ antibodies on the surface of the microtiter plates act as capture antibodies.

The plasma samples from the HUMAN BLOOD STIMULATION TUBES and the standards are pipetted into the wells of the microtiter plate. A biotin-conjugated secondary antibody (detection antibody solution) is then added, and the microtiter plate is incubated. If IFN-γ is present in the sample, it binds to the capture antibodies immobilised on the microtiter plate.

After the first incubation step, the plate is washed with a wash buffer to remove unbound material. Following the washing step, the conjugate solution is pipetted into the wells of the microtiter plate, and the plate is incubated again. After a final washing step, the substrate solution is added, which induces a more or less intense blue colour change. The colour change is interrupted by adding the stop solution and the colour switches from blue to yellow.

Wells that do not contain IFN-γ remain colourless. The change in colour is measured using an ELISA reader at a wavelength of 450 nm. The concentration of IFN-γ is directly proportional to the intensity of the colour. Because the sample are measured parallel with the standards, the IFN-γ concentration can be determined quantitatively for each sample.

Materials Provided with the Kit

HUMAN IFN-γ ELISA:

Component	Symbol Cap colour	Quantity	
	Σ REF	22 Tests LIO-Feron 02_22	44 Tests LIO-Feron 02_44
Microtiter plate strips (8 wells each) coated with monoclonal anti-human IFN-γ capture antibody	MTP	12 strips (≙ 1 plate)	24 strips (≙ 2 plate)
Recombinant human IFN-γ standard (12,5 IU, lyophilised)	STA ■	2x	4x
Detection Antibody Solution with monoclonal anti-human IFN-γ detection antibody, biotin-conjugated, contains casein	DET ■	7 mL	14 mL
Conjugate Solution with streptavidin, HRP- conjugated	CON ■	14 mL	24 mL
Substrate Solution, contains 3,3',5,5'-Tetramethylbenzidin (TMB)*	TMB ■	14 mL	24 mL
Stop Solution, contains H_2SO_4*	STO □	7 mL	14 mL
Wash Buffer (10x concentrate)	WB ■	100 mL	2x 100 mL
Incubation Buffer, contains bovine serum albumin	IB ■	40 mL	40 mL
Instructions for use		1x	1x

* Read the "*Warnings and Precautions*" section.

Controls and Calibrators

The recombinant human IFN-γ standard contained in the kit has been tested against a standard of the NIBSC (REF: 82/587). The standard serves as internal quality control (see section *"Quality Control of the Test Result"*).

Materials Required but not Provided

- LIOFeron® TB/LTBI HUMAN BLOOD STIMULATION TUBES (REF LIO-Feron 01_22)
- Container for dilution of the standards e.g. sterile 1.5 mL plastic vials and (optional) for plasma collection and storage
- Incubator 37 °C (± 0.5 °C), CO_2 not necessary
- Centrifuge, suitable for blood collection tubes (RCF range from 2000 to 3000)
- Vortex mixer
- Deionised or distilled water
- Beakers, glass bottles and/or measuring cylinders for preparing the 1x Wash Buffers
- Calibrated microlitre pipettes and disposable tips, suitable for 50 µL to 1000 µL
- Automated microtiter plate washer (96 well, recommended) or calibrated multichannel pipettes, suitable for 50 µL to 400 µL with disposable tips (optional)
- Timer
- Microtiter plate reader (450 nm)

Time Required for the Test Procedure

The least amount of time from sample collection to result is approximately 20 hours. The majority that time frame is needed for the incubation of the HUMAN BLOOD STIMULATION TUBES. It is recommended, if possible, to perform various test at the same time, as to reduce the average time required per test.

HUMAN BLOOD STIMULATION TUBES

Pipetting of the samples into the tubes:	approx. 10 minutes per sample
Incubation time:	16 to 24 hours (37 °C ± 0.5 °C)
Collection of plasma samples:	approx. 5 minutes

HUMAN IFN-γ ELISA

For one microtiter plate:	Approx. 3 hours (22 tests, < 1 h lab work) additionally, approx. 15 minutes for each additional microtiter plate

Stability and Storage

Storage Conditions of the Kit Components

Store the kits or the individual components in accordance with the temperatures specified on the respective labels. Never freeze the test components or expose them to temperatures above 30 °C. Mind the expiry dates printed on the labels. Do not use expired or incorrectly stored components. Do not use damaged or unsealed components.

Store the ELISA Kit at 2 – 8 °C.

Protect the TMB Substrate Solution from direct light.

Storage and Handling of Samples

For notes and information on storage and handling of samples, please refer to the section "*Sample Collection and ELISA Preparation*".

Opened Components and Reconstituted Standards

The MTP microtiter plate is sensitive towards humidity. After first opening, the stability of the microtiter plate is given up to 3 months if the remaining strips are stored in a closed aluminium bag at 2 – 8 °C with a desiccant bag. The individual microtiter plate strips are single use only.

After diluting the WB Wash Buffer (10x concentrate), the diluted solution (1x Wash Buffer) may be used for up to 1 month at 2 – 8 °C.

The reconstituted standard STA stock solution is stable for up to 2 months at 2 – 8 °C. Note the reconstitution date on the label of the standard.

Liquid kit components may be stored for up to 3 months after their first opening if the bottles are tightly closed after each use.

NOTE: Test results are unaffected by precipitates appearing in the CON Conjugate Solution. Visible bubbles in the wells of the unused microtiter plate do not affect test results.

Warnings and Precautions

Only for in vitro diagnostic use! Not for self-testing!

- Follow the instructions for test procedure and interpretation of results carefully!
- In accordance with Good Laboratory Practice (GLP) all laboratory devices used should be maintained regularly and calibrated for accuracy and precision.
- Do not ingest or swallow! Do not eat, drink or smoke in the laboratory! Always wear protective clothing while working (disposable gloves, safety goggles and lab coat)! Avoid contact of kit reagents with skin, eyes or mucosa.
- Only use reagents within their expiry period (printed on the labels).
- Bring all kit components to room temperature (preferably 15 – 30 °C). Mix the liquid kit components before use by careful swaying. Immediately store the test kit after use at 2 – 8 °C. The test is sensitive towards temperatures above 30 °C.
- Only use fresh blood samples containing lithium heparin as anticoagulant. Other body fluids than blood with lithium heparin are not validated and can yield incorrect results! Store blood samples at room temperature (preferably 15 – 30 °C)! Do not store blood samples below 15 °C! Only use the blood samples within 16 hours of the venipuncture.
- Assay the standards at least in duplicate. The human plasma samples can be analysed by single determination.
- Do not use reagents from different kits or batches and do not mix reagents of different kits or batches.
- Avoid contamination of reagents. Do not use the same container for several samples!

- To avoid contamination of the samples, work under aseptic conditions. Avoid usage of turbid samples, as those samples might be contaminated with bacteria. Only use the clear supernatant for IFN-γ determination. Avoid contamination with red blood cells while collecting the supernatant. Separate the red blood cells from the plasma by centrifugation if necessary.
- Avoid repeated freezing and thawing of the plasma samples as it could lead to denaturation of IFN-γ.
- Before pipetting, thoroughly mix all reagents by gentle tilting or swaying. Do not shake vigorously to avoid foam formation. Pipette with constant intervals to ensure equal conditions in all wells of the microtiter plate.
- Avoid touching the inside of the screw caps to reduce risk of contamination. Single use only. Do not use if vials are damaged or opened.
- Protect the TMB Substrate Solution from direct light.
- If serious incidents occur with the kit (see Article 2 No. 68 of Regulation (EU) 2017-746 IVDR), immediately report this to the manufacturer and your local competent authority.

For more information, please contact sales@lionex.de per e-mail.
MSDS are available for download at www.lionex.de.

ATTENTION:
Treat human blood and plasma as potentially infectious!

Dispose of leftover human blood and plasma samples as well as materials that have come into contact with them in accordance with local regulations.

Hazards and Precautions

TMB Substrate Solution

Contains: 2-Pyrrolidone. Warning! Danger!

H319 causes serious eye irritation.

H360 May damage fertility or the unborn child.

Disposal in accordance with official regulations.

When exposed: Get medical advice / Get medical attention.

Obtain special instructions before use.

Wear protective gloves / protective clothing / safety goggles / face protection.

STO Stop Solution

Contains: Sulfuric acid. Warning!

H290 May be corrosive to metals.

H315 Causes skin irritation.

H319 Causes serious eye irritation.

Disposal in accordance with official regulations.

When exposed: Get medical advice / Get medical attention.

Obtain special instructions before use.

Wear protective gloves / protective clothing / safety goggles / face protection.

Sample Collection and ELISA Preparation

LIOFeron® TB/LTBI works best with fresh blood samples.

A minimum of 4.5 mL human blood is required. Collect the sample under standard laboratory conditions (aseptic, avoid haemolysis) using lithium heparin blood collection tubes. Venipuncture should only be performed by appropriately qualified personnel. Comply with legal regulations and requirements for blood collection.

If the blood sample cannot be applied to the HUMAN BLOOD STIMULATION TUBES immediately after blood collection, the whole blood can be stored up to 16 hours at 15 – 30 °C.

Use and Storage of Stimulated Plasma

It is recommended to pipette the plasma directly after centrifugation of the tubes onto the ELISA plate and perform the HUMAN IFN-γ ELISA.

If the HUMAN IFN-γ ELISA cannot be performed immediately after stimulation, the tubes can be stored up to 4 days at 2 – 8 °C.

For longer storage, separate the plasma from the red blood cells and store at 2 – 8 °C (up to 28 days). However, this may lead to a decreased IFN-γ concentration. Separated plasma samples can be stored for a longer period at below -20 °C. Before use, frozen samples must be thawed and thoroughly mixed. Avoid repeated freezing and thawing of samples!

Preparation of Reagents

WB Wash Buffer (10x concentrate):

Bring the WB Wash Buffer (10x concentrate) to room temperature (preferably 15 – 30 °C) and mix the buffer carefully before use.

If salt crystals have formed in the bottle, heat the concentrate for approximately 15 minutes at 37 °C.

Dilute with distilled water (1 + 9 volumes) before use, e.g. 100 mL WB Wash Buffer (10x concentrate) + 900 mL distilled water.

STA Recombinant human IFN-γ Standard (lyophilised):

Reconstitute the IFN-γ standard by adding the on the label indicated volume of IB Incubation Buffer into the vial to get a stock solution with 12.5 IU/mL. Thoroughly mix the stock solution after adding the Incubation Buffers for at least 10 seconds (e.g. with a vortex mixer) until the protein is fully solubilized. It is recommended to let the vial rest for 10 – 30 minutes at room temperature afterwards.

Prepare the standard solutions S1 – S4 following the pipetting scheme depicted on the following page (Figure 1, Table 1).

Table 1: Preparation of the standard solutions S1 - S4

Standard solution No.	conc. IFN-γ	Volume of stock solution or standard solution	Volume Incubation Buffer	Total volume
S1	**4 IU/mL**	80 µL of stock solution	170 µL	250 µL
S2	**1 IU/mL**	50 µL standard solution S1	150 µL	200 µL
S3	**0.25 IU/mL**	50 µL standard solution S2	150 µL	200 µL
S4 (Blank)	**0 IU/mL**	-	150 µL	150 µL

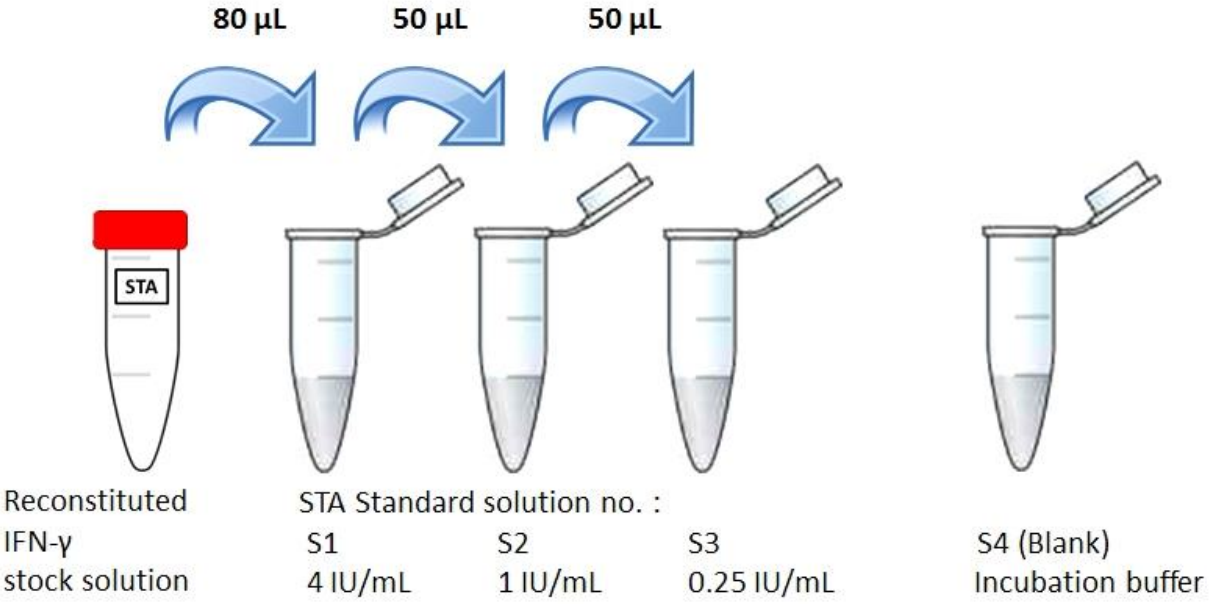


Figure 1: IFN-γ concentrations of the prepared standard solutions S1 – S3

Test Procedure

HUMAN BLOOD STIMULATION TUBES

The test procedure requires approx. 10 minutes for each sample.
Incubation time: 16 - 24 hours

1. Take the required amount of HUMAN BLOOD STIMULATION TUBES NC, TB A, TB B and PC from the kit. Place the tubes upright in a suitable rack. Label the HUMAN BLOOD STIMULATION TUBES appropriately, e.g. with a sample number or ID. Remove the cap by pulling up (no screwing) and place the cap inside up on a clean, flat surface.
2. Mix the blood sample in the lithium heparin blood collection tube by carefully inverting the tube (approx. 10 times) before each pipetting step. Pipette 1 mL of the blood sample into each HUMAN BLOOD STIMULATION TUBE (NC, TB A, TB B und PC) and close them. Attention! There are 2 closure points. Push the cap completely down!
3. Mix each HUMAN BLOOD STIMULATION TUBE filled with 1 mL blood by gently inverting 10 times. Avoid vigorous shaking to prevent haemolysis of the blood cells!
4. Place the rack with the HUMAN BLOOD STIMULATION TUBES (filled with 1 mL lithium heparin blood each) upright in an incubator at 37 °C (± 0.5 °C). Incubate the tubes for at least 16 hours. The maximum incubation period is 24 hours.

5. Finally, remove the rack with the HUMAN BLOOD STIMULATION TUBES from the 37 °C incubator (without shaking!). It is possible to harvest the plasma without centrifugation but pay attention not to contaminate the plasma sample with red blood cells. The red blood cells are separated from the plasma by the gel.

   Important note: If the plasma is contaminated with red blood cells, centrifuge the tube for 15 minutes at 2000 to 3000 RCF (g) before harvesting the plasma. After centrifuging, avoid pipetting up and down or mixing the plasmas by any means. Always ensure that the plasma does not mix with the red blood cells on the surface of the gel.

6. The plasma samples should only be harvested using a pipette. The plasma can be transferred directly from the HUMAN BLOOD STIMULATIONS TUBES to the HUMAN IFN-γ ELISA plate. Pipette 50 µL of the clear supernatant from the HUMAN BLOOD STIMULATION TUBES into the wells of the microtiter plate and continue with step 5 of the "*HUMAN IFN-γ ELISA Test Procedure*".

   Note: It is possible, to use an automated ELISA-Workstation.

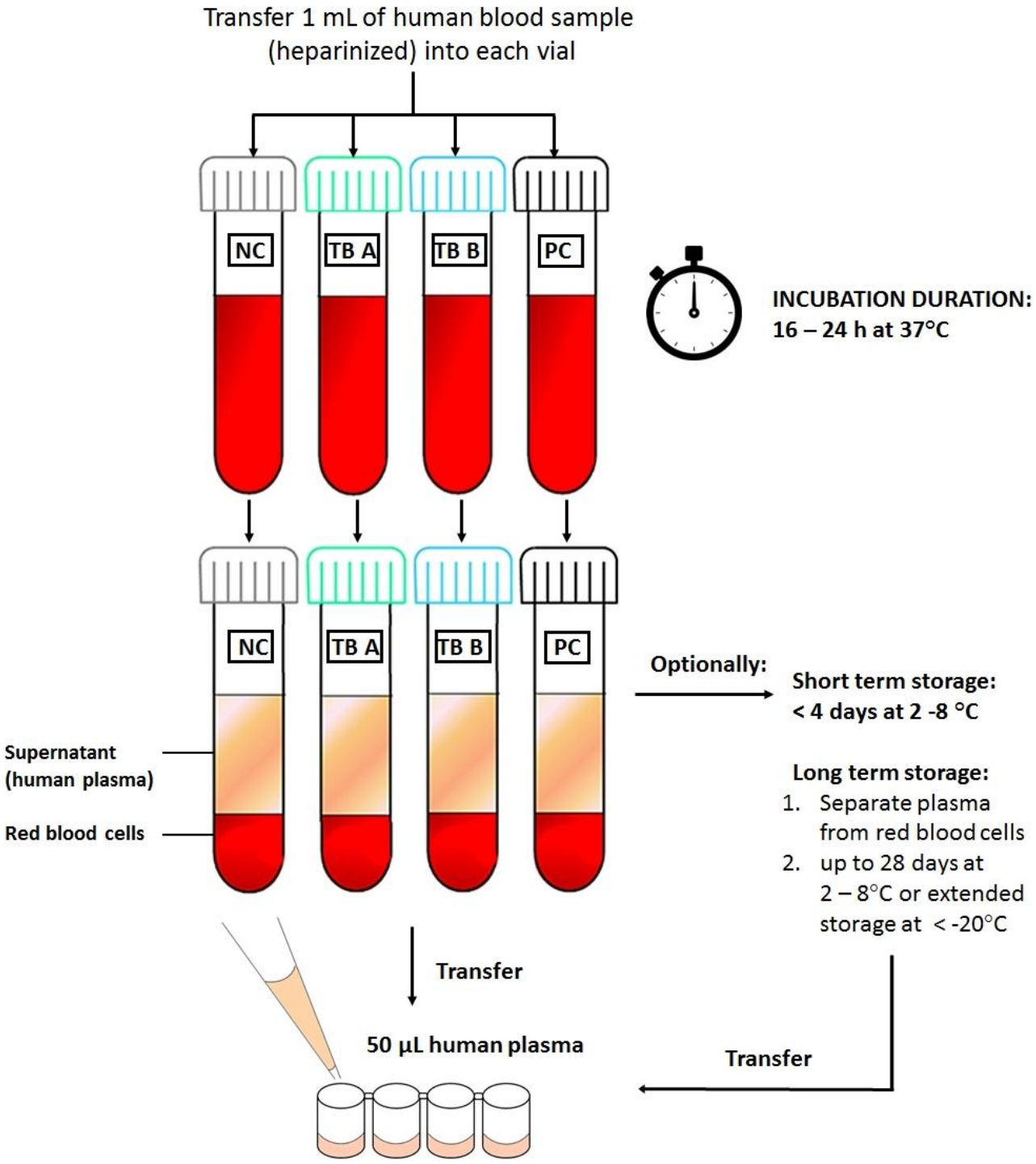


Figure 2: Quick guide for using the HUMAN BLOOD STIMULATION TUBES

HUMAN IFN-γ ELISA

The test procedure requires approx. 3 hours.

1. Have the prepared Wash Buffer and standard solutions from the section "*Preparation of Reagents*" ready.

2. Equilibrate all kit components to room temperature (15 – 30 °C, approx. 30 minutes).

3. Remove the MTP Microtiter plate from the aluminium bag and insert the required microtiter strips into the holder provided. Put any unused microtiter strips back into the aluminium bag.

4. Pipette 50 µL of each sample, 50 µL of the prepared standard solutions No. S1 - S3 and 50 µL of the IB Incubation Buffer as blank (S4) into the corresponding wells. The standards and the blank must be assayed in duplicate; the samples may be measured as single determination.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 NC	3 NC	5 NC	7 NC	9 NC	S1		13 NC	15 NC	17 NC	19 NC	21 NC
B	1 TB A	3 TB A	5 TB A	7 TB A	9 TB A	S2		13 TB A	17 TB A	17 TB A	19 TB A	21 TB A
C	1 TB B	3 TB B	5 TB B	7 TB B	9 TB B	S3		13 TB B	15 TB B	17 TB B	19 TB B	21 TB B
D	1 PC	3 PC	5 PC	7 PC	9 PC	S4 (Blank)		13 PC	15 PC	17 PC	19 PC	21 PC
E	2 NC	4 NC	6 NC	8 NC	10 NC	11 NC	12 NC	14 NC	16 NC	18 NC	20 NC	22 NC
F	2 TB A	4 TB A	6 TB A	8 TB A	10 TB A	11 TB A	12 TB A	14 TB A	16 TB A	18 TB A	20 TB A	22 TB A
G	2 TB B	4 TB B	6 TB B	8 TB B	10 TB B	11 TB B	12 TB B	14 TB B	16 TB B	18 TB B	20 TB B	22 TB B
H	2 PC	4 PC	6 PC	8 PC	10 PC	11 PC	12 PC	14 PC	16 PC	18 PC	20 PC	22 PC

Figure 3: Example for a pipetting scheme for the HUMAN IFN-γ ELISA; standard solutions (S1 - S3) and blank (S4) as duplicate determination; samples No. 1 - 22 as single determination from the HUMAN BLOOD STIMULATION TUBES (NC, TB A, TB B und PC)

Note: The pipetting scheme can be chosen freely, but it is recommended to use the example scheme from Figure 3.

5. Add 50 µL of the DET Detection Antibody Solution in each well. Cover the plate with a microtiter plate lid. Mix the standards/samples thoroughly using a microtiter plate shaker for at least 60 seconds at 500 bis 1000 rpm. Then let the plate incubate.

   INCUBATION PERIOD: 1 hour (± 5 minutes) at 15 – 30 °C

6. Washing: Empty the wells of the plate (dump or siphon) and wash with 6x 400 µL diluted Wash Buffer per well.

7. Pipette 100 µL of the CON Conjugate Solution into each well and incubate afterwards.

   INCUBATION PERIOD: 1 hour (± 5 minutes) at 15 – 30 °C

8. Washing: Empty the wells of the plate (dump or siphon) and wash with 6x 400 µL diluted Wash Buffer per well.

9. Pipette 100 µL of the TMB Substrate Solution into each well and incubate afterwards.

   INCUBATION PERIOD: 30 minutes (± 1 minute) at 15 – 30 °C in the dark

10. Rapidly add 50 µL of the STO Stop Solution per well to the Substrate Solution to terminate the substrate reaction. **Attention!** Do not wash!

11. Measure the absorption (OD) at 450 nm (optional reference wavelength: 620 nm). The colour is stable for at least 60 minutes.

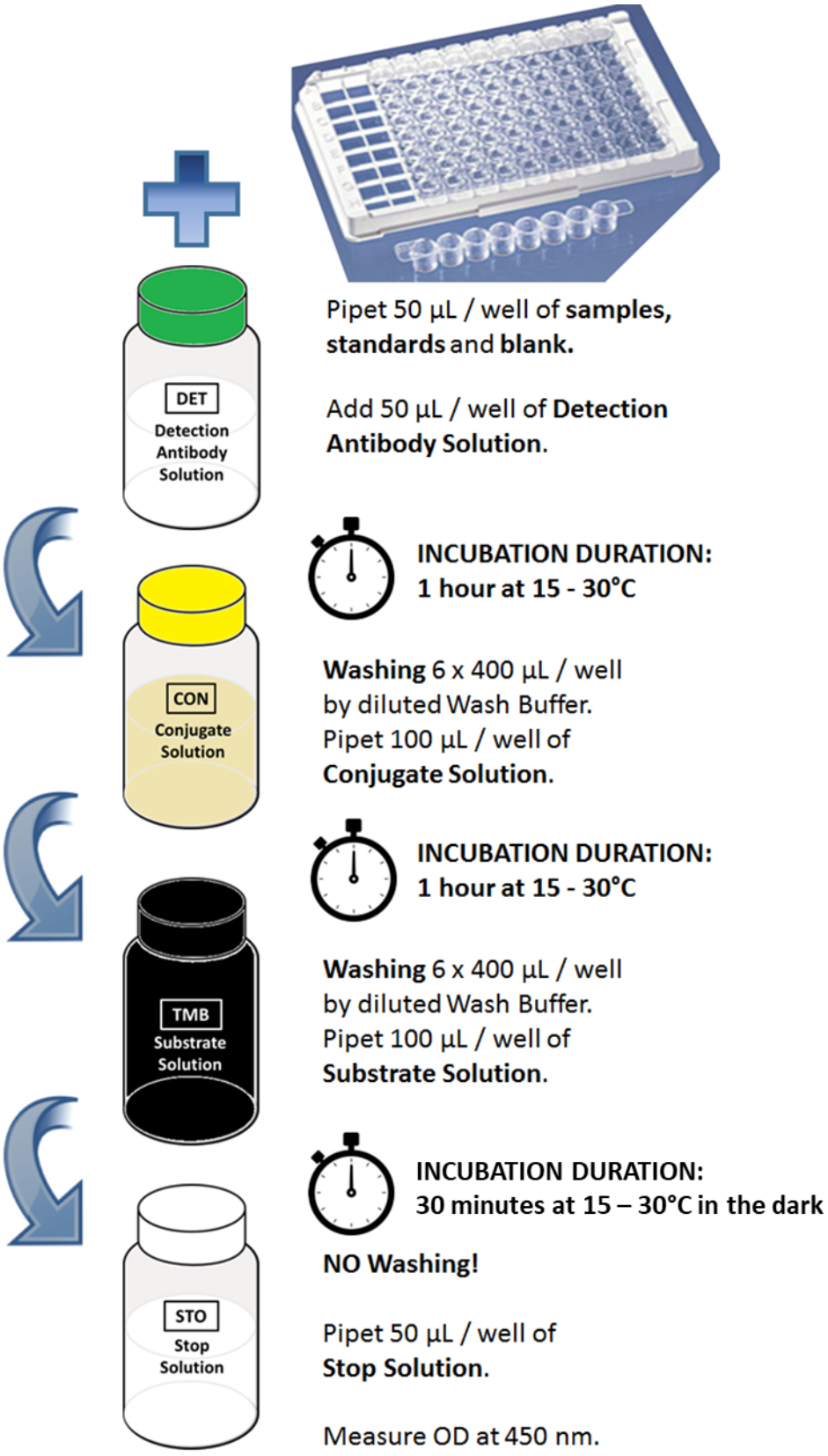


Figure 4: Schematic depiction of the test procedure of the HUMAN IFN-γ ELISA

Calculation and Interpretation

We recommend using automatic computer programmes to calculate the reference curve. These calculations can be carried out using statistical software such as Microsoft® Excel®. We recommend using this software to calculate the regression analysis, the coefficient of variation CV (%) for the standards and the coefficient of determination (R^2) or the correlation coefficient (R) of standard curve.

Generation of the Standard Curve using an x-y diagram

Calculate the mean OD values for the measured absorptions for each Standard Solution S1 - S3 and the blank (S4).

Create a ln-ln standard curve by plotting the natural logarithm ($\log_e$ or ln) of the mean OD values of the Standard Solutions S1 - S3 on the vertical axis (y-axis) against the natural logarithm of the IFN-γ concentration ($c_{IFN-\gamma}$) of the Standard Solutions on the horizontal axis in IU/mL (x-axis). Do not take the zero standard (blank) into account for this step.

Calculate the straight line for the standard curve by using a regression analysis. Use the standard curve to determine the IFN-γ concentration (IU/mL) for each of the plasma samples using the OD value of the respective sample. In an x-y graph, the trend line corresponds to the standard curve and the corresponding function and coefficient of determination can be displayed in the diagram.

NOTE: The difference between individual values for S1 – S4 should not exceed 10%.

Table 2: Example of the conversion steps for calculating the standard curve

Standard	Concentration [IU/mL]	ln($c_{IFN-\gamma}$)	OD value	Mean OD value	ln(OD)
S1	4	1.386	3.051 3.114	3.0825	1.126
S2	1	0	1.036 1.085	1.0605	0.059
S3	0.25	-1.386	0.293 0.311	0.302	-1.197

Calculating the IFN-γ Concentration from the OD Values

The functional equation of the standard curve should have the following form:

$$y = a \times x + b \qquad (1)$$

Where x corresponds to the natural logarithm of the IFN-γ concentration and y corresponds to the natural logarithm of the OD values.

$$\ln(OD) = a \times \ln(c_{IFN-\gamma}) + b \qquad (2)$$

In order to calculate the IFN-γ concentration from the measured OD values using this equation, the equation must be rearranged accordingly for x or respectively the concentration.

$$x = e^{\frac{\ln(y)-b}{a}} \quad bzw. \quad c_{IFN-\gamma} = e^{\frac{\ln(OD)-b}{a}} \qquad (3)$$

To quantify the amount of IFN-γ secreted be the antigen stimulated blood cells, the calculated values for tubes TB A TB A and TB B TB B as well as the positive control PC must be adjusted for the value of the negative control NC (see Table 3). For test interpretation, the 25% value of the negative control result needs to be calculated as well.

Table 3: Example calculation for obtaining the adjusted IFN-γ concentration

Sample	OD value	IFN-γ concentration	IFN-γ concentration adjusted
NC	0.206	0.153	
TB A	1.511	1.381	1.228
TB B	1.558	1.440	1.287
PC	1.744	1.680	1.527

Quality Control of the Test Result

The HUMAN IFN-γ ELISA contains an internal control. The standard solutions are considered as internal procedural control. It confirms the correct execution of the rest procedure. The test result depends on the generation of the standard curve. The measured values for the standards and the standard curve generated from them must fulfil the following criteria:

- Mean OD of S1 must be > 0.60
- CV (%) of replicate OD values for S1 and S2 must be < 15%
- Variation of replicate OD values for S3 and S4 (blank) < 0.04
- Coefficient of determination (R^2) for the standard curve > 0.9604 or correlation coefficient (R) > 0.98
- Mean OD of S4 (blank) is an internal negative procedural control and should be < 0.19

If these criteria are not met, the analysis of the test results will be affected, and the test will be invalid. Insufficient sample volume or incorrect execution of the

test procedure are the most likely reasons why the required criteria are not met.

If the above-mentioned limit of S4 is exceeded, the washing procedure should be improved and/or the room temperature should be controlled during the test (the room temperature should be 15 – 30 °C).

Review the sample preparation and test procedure instructions and troubleshooting guide again and repeat the test using a new microtiter plate strip. If the problem persists, contact the manufacturer or your distributor.

Interpretation of the Results

The data for evaluation of the LIOFeron® TB/LTBI ELISA are displayed in Table 4. A schematic procedure for interpreting the test result is shown in Figure 5. Note that the value of the negative control must be subtracted from the values for TB A, TB B and the positive control.

Table 4: Interpretation of test results

Result	Control -	TB A	TB B	Control +
	Negative control [IU/mL]	TB A minus negative control [IU/mL]	TB B minus negative control [IU/mL]	Positive control minus negative control [IU/mL]
Positive	≤ 8.00	TB A and/or TB B (other not relevant) ≥ 0.35 and ≥ 25% of negative control value		not relevant
Negative	≤ 8.00	≥ 0.35 and < 25% of negative control value	≥ 0.35 and < 25% of negative control value	≥ 0.50
		< 0.35	≥ 0.35 and < 25% of negative control value	
		≥ 0.35 and < 25% of negative control value	< 0.35	
		< 0.35	< 0.35	
Indeterminate	≤ 8.00	≥ 0.35 and < 25% of negative control value	≥ 0.35 and < 25% of negative control value	< 0.50
		< 0.35	≥ 0.35 and < 25% of negative control value	
		≥ 0.35 and < 25% of negative control value	< 0.35	
		< 0.35	< 0.35	
	> 8.00	not relevant	not relevant	not relevant

The readings for the positive control PC may be outside the upper limit range of the microtiter plate reader. This does not affect the test result.

Individuals with negative control NC values greater than 8 IU/mL are classified as indeterminate, as a 25% higher TB antigens response may be outside the assay measurement range.

For 'Indeterminate' results, refer to the "*Troubleshooting Guide*" section to identify possible causes.

IGRA test results alone are not sufficient to diagnose TB infection and should always be considered in combination with the individual's clinical status, the results of other diagnostic tests and the epidemiological background information.

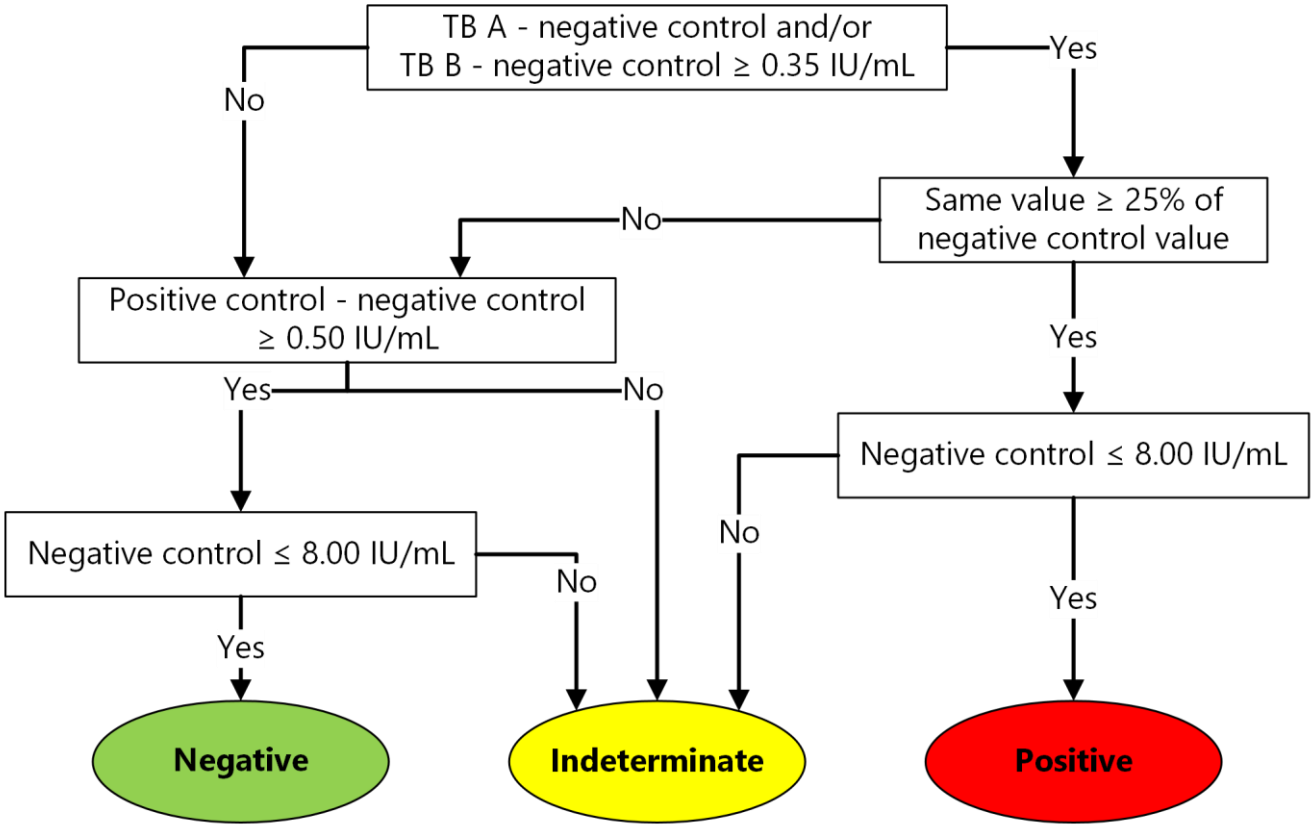


Figure 5: Interpretation scheme for the LIOFeron® TB/LTBI ELISA

Limitations

Follow the instructions of the test procedure and interpretation of the results carefully! Insufficient sample volume or incorrect handling of the test procedure are the most likely reasons for not meeting the required QC criteria (see section "*Quality Control of the Test Result*").

IGRAs should be used to aid in the diagnosis of an infection with *M. tuberculosis*. A positive test result indicates that an infection with *M. tuberculosis* is likely. A negative result indicates that an infection with *M. tuberculosis* is unlikely. An indetermined result requires repeat testing or alternative testing.

The results of the LIOFeron® TB/LTBI test should always be considered in combination with the clinical status of the individual, the results of other diagnostic tests and the epidemiological background information.

Atypical cases may occur when infection with *M. tuberculosis* is unlikely, and the prevalence of NTM is exceptionally high.

The test was developed for the detection of human IFN-γ in plasma (lithium heparinized). The test has not been validated for detection of IFN-γ in body fluids other than human plasma and may produce false results.

The blood sample should be stored at room temperature (15 – 30 °C) after collection and not be used if the samples is older than 16 hours after venipuncture. Do not freeze the blood sample. The vitality of the cells cannot be guaranteed after incorrect or prolonged blood storage.

Only the clear supernatant (human plasma) should be used for IFN-γ measurements. Avoid contamination by red blood cells while harvesting the human plasma. If necessary, separate the red blood cells from the plasma by centrifuging. Avoid bacterial contamination of the samples!

The standard curve should not be extrapolated if the calculated values are non-linear. Samples with absorbance values exceeding the highest standard concentration should be re-analysed at higher dilution. For further analysis the human plasma samples can be diluted with IB Incubation Buffer, e.g. 1:20 (50 µL human plasma in 950 µL IB Incubation Buffer).

A high circulating IFN-γ level in human blood can interfere with the test and lead to indeterminate results. Reversion and conversions can occur in IGRA tests. Various factors can affect reversion including medications. Dynamic changes may occur which are not associated with the clinical conditions of the patients, when serially repeated. These variations, also observed in patients treated with biological agents, hardly ease the interpretation of the results. A careful clinical judgment is still required to address treatment decisions in immunocompromised patients undergoing serial IGRAs[28].

Technical Information

Indeterminate Results

If the test result is indeterminate, this may relate to the immune status of the tested patients, but this may also be due to technical factors:

- The storage conditions regarding temperature for the blood samples was neglected (recommended: 15 – 30 °C).
- The HUMAN BLOOD STIMULATION TUBES were insufficiently shaken after filling (gently invert at least 10x).
- The time between venipuncture and incubation of the blood-filled HUMAN BLOOD STIMULATION TUBES at 37 °C was too long (> 16 hours).
- Incorrect washing during the HUMAN IFN-γ ELISA.
- It is known that the rate of 'Indeterminate' results in immunocompromised patients can be higher than in other groups[26].

Clotted Human Plasma

During prolonged storage of plasms samples, fibrin proteins produce clots, which should be sedimented by centrifuging the plasma samples to facilitate pipetting of the required plasma volumes.

Interfering Substances

Only use fresh blood samples with lithium heparin as anticoagulant. The test has not been validated for detection of IFN-γ in body fluids other than human plasma and may produce false results (e.g. citrate blood inhibits assay performance).
Analytical specificity was determined by measuring potential interfering substances. Substances that could be used to treat the patient, substances that can be ingested by the patient, and substances that could be present in certain sample types were considered. The following substances, tested in the concentrations stated below, did not affect the test results:

Acetaminophen	20 mg/dL
Acetylsalicylic acid	20 mg/dL
Ascorbic acid	20 mg/dL
Bilirubin	60 mg/dL
Caffeine	20 mg/dL
Glucose	2000 mg/dL
Haemoglobin	500 mg/dL
Lithium-Heparin	3 mg/dL
Tetracycline	20 mg/dL
Sodium-Heparin	3 mg/dL

Haemolyzed samples should not be used because red blood cell components are known to migrate into the plasma and potentially interfere with the test result.

An excessive amount of lipids in a sample can cause physicochemical inhomogeneity. Furthermore, a high concentration of lipids can potentially alter the binding behaviour of cytokines and thus distort the results. Therefore, lipemic samples must not be used.

Recent or ongoing treatment of TB may lead to incorrect results. Cytokine level in the blood can diminish rapidly after treatment and be so low in the patient's blood samples that no elevated cytokine level can be detected even in the case of infection or illness.

Please note that false-positive test results may be caused by interference from heterophile antibodies. Heterophile antibodies are commonly present in human plasma from subjects with various diseases that cannot be detected with this test. Sodium azide and other nucleophilic substances (e.g., used as preservatives for various buffers) interfere with the activity of horseradish peroxidase. Therefore, avoid using wash buffers or other solutions that may contain such interfering substances. Unreliable or indeterminate results may occur due to extrapolation or deviations from the procedure.

Performance Characteristics

Measuring Range of the HUMAN IFN-γ ELISA

Limit of quantitation (LoQ), limit of detection (LoD) and limit of blank (LoB) of the LIOFeron® TB/LTBI were calculated from blank values (23 repetitions, different batches and operators on different days). The following values were calculated from the measured data:

LoQ = 0.2860 IU/mL LoD = 0.1360 IU/mL LoB = 0.0936 IU/mL

Comparative studies

To determine the concordance of LIOFeron® TB/LTBI with the reference method (QuantiFERON®TB-Gold Plus (Qiagen)), 135 samples were tested. The concordance of LIOFeron® TB/LTBI with the reference method was 96.00% for the positive group and 98.82% for the negative group (Table 5).

Table 5: Comparative study LIOFeron® TB/LTBI versus QuantiFERON®TB-Gold Plus (Qiagen)

Method		QuantiFERON®TB-Gold Plus*	
		positive	negative
LIOFeron® TB/LTBI	negative	2	84
	positive	48	1
Total results:		50	85
Concordance:		96.00 %	98.82 %

*Qiagen

Sensitivity and Specificity

The analytical sensitivity was determined by measuring spiked samples with different concentrations of IFN-γ within the same assay (5 replicates each). It was measured to be about 0.0625 IU/mL.

Clinical Sensitivity and Specificity

Where possible, the LIOFeron® TB/LTBI results were also compared with the final diagnosis. A total of 294 samples with a known diagnosis were considered. The concordance among negative samples was 97.61% (healthy individuals, HIV, and NTM patients, 251 samples). The concordance among positive samples was 97.67% (TB and LTBI, 43 samples) (Table 6).

Table 6: Summary table for the results of LIOFeron® TB/LTBI compared to clinical diagnosis

Sample group	positive	negative	Total number	Concordance
positive (TB + LTBI)	42	1	43	97.67 %
negative (HIV, NTM+, healthy)	6	245	251	97.61 %

Abbreviations: TB = tuberculosis, NTM = nontuberculous mycobacteria, LTBI = latent TB

Assay Performance

Accuracy: To demonstrate the linearity of LIOFeron® TB/LTBI, 17 samples spiked with different concentrations of IFN-γ (plasma pool) were randomly placed on the microtiter plate together with the unspiked plasma pool (= zero plasma) (5 replicates each). The calculated results (IU/mL) were plotted against the expected results. Calculation of the correlation coefficient R (R = 0.9953, Figure 6) confirmed that the

assay is linear up to 4 IU/mL. For concentrations up to 8.0 IU/mL, the assay was approximately linear (R = 0.9824).

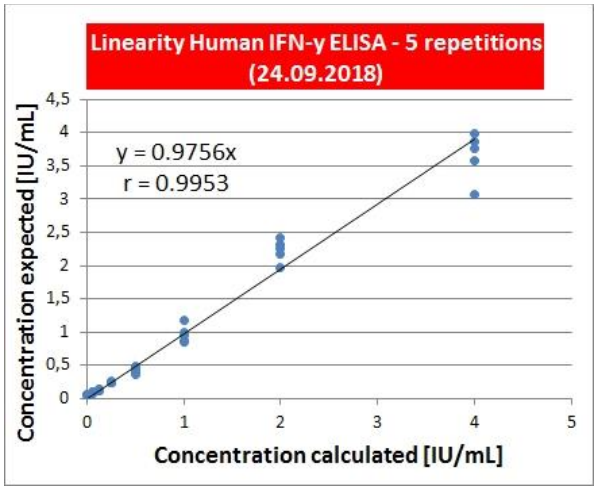


Figure 6: LIOFeron® TB/LTBI: Proof of linearity between 0 and 4 IU/mL

High dose hook effect: Human plasma was spiked with IFN-γ in concentrations up to 10,000 IU/mL and measured with HUMAN IFN-γ ELISA following the procedure in the instructions for use. No high dose hook effect was observed for concentrations up to 10,000 IU/mL.

Precision: To determine reproducibility, intra- and inter-assay variations as well as inter-operator variations and batch-to-batch variations were determined by measuring samples with different reactivity.

Inter-assay variation: Determined by repeated measurements of 3 samples with different reactivity by 3 different batches on 10 different days (negative, weak positive and positive sample). The inter-assay imprecision (CV (%)) was 11.73% for the weak positive sample and 11.9% for the positive sample. For the negative sample (= zero sample) the imprecision was 27.39%. This high degree of variation was expected due to the low level of IFN-γ concentration. The Variation around a low concentration is usually greater than at higher concentrations.

Intra-assay variation: Determined by repeated measurements of samples with different reactivity (3 samples with different reactivity: negative, weak positive, and positive sample; 20 replicates). The intra-assay uncertainty ranged between 4.35% and 10.11% (CV (%)).

Batch-to-batch variation: Determined by measuring various samples with three different batches. The imprecision ranged from 2.52% to 19.05%. As expected, a higher CV (%) of more than 10% was observed for the lower concentration samples. The imprecision is known to be higher at low concentrations than at higher concentrations. The mean CV (%) calculated for all samples was 8.37%.

Inter-operator variation: 53 samples with varying reactivity were measured by three different operators on different days. Operator-related deviations between the measured OD values ranged from 1.465% to 31.673%. For low values (IU/mL <0.35), the mean CV (%) was 22.195%. For values above the cutoff (IU/mL >0.350 <8.0), the mean CV (%) was 12.759%.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving problems which could occur. If the problem persists, contact the manufacturer or your local distributor.

Table 7: HUMAN IFN-γ ELISA Troubleshooting Guide

Nonspecific colour development or high background	
Possible reason	**What to do**
Incomplete washing of microtiter plate	Wash the plate at least 6 times with 400 µL diluted WB Wash Buffer (1x) per well.
Expiry date of the kits or one of its components has passed	Use the kit components before the expiry date. Pay attention to the expiry of reconstituted STA Standard Solutions and opened MTP Microtiter plates (see "Stability and Storage").
Incubation temperature is too high	The ELISA should be incubated at room temperature (15 – 30 °C).
Mixing or dilution omitted	Ensure each step is done correctly. Recheck the sample preparation and test procedure instructions and repeat the test using a new microtiter plate strip.
Cross contamination	Be careful when pipetting samples and solutions. Do not use TMB Substrate Solution that appears bluish and dispose of properly. Make sure to use clean reagent and sample containers. Do not mix the human plasma after centrifuging the HUMAN BLOOD STIMULATION TUBES to avoid contamination of the sample with the gel.

Low OD values of the standards	
Possible reason	**What to do**
Pipetting error	Pipettes should be calibrated and used according to the manufacturer's instructions.
Expiry date of the kits or one of its components has passed	Use the kit components before the expiry date. Pay attention to the expiry of reconstituted STA Standard Solutions and opened MTP Microtiter plates (see "Stability and Storage").
Incubation temperature is too low	The ELISA should be incubated at room temperature (15 – 30 °C).
Dilution error when preparing the standards	Ensure that the STA Standard Solutions are prepared according to the section "*Preparation of Reagents*"
Incubation period too short	The incubation period of the mixture of plasma samples + DET Detection Antibody Solution is 60 minutes. The incubation period of the CON Conjugate Solution is 60 minutes as well. The incubation period of the TMB Substrate Solution is 30 minutes.
Reagents are too cold	All reagents should be brought to room temperature (15 – 30 °C) before use.
Wrong filter used in the reader	The plate should be read at 450 nm (optional reference wavelength: 620 nm) within 60 minutes after adding the STO Stop Solution.

Coefficient of determination of the standard curve too low or high variation of duplicate determinations	
Possible reason	**What to do**
Dilution error when preparing the standards	Ensure that the STA Standard Solutions are prepared according to the section "*Preparation of Reagents*"
Incomplete washing of microtiter plate	Wash the plate at least 6 times with 400 µL diluted WB Wash Buffer (1x) per well.
Insufficient mixing	During reconstitution, vortex the STA Standard stock solution for 10 seconds. Ensure that standard solutions S1 - S3 are thoroughly mixed before the next dilution step and adding them to the microtiter plate.
Non continuous pipetting technique or interruption of the test	Pipetting of sample and standard solutions should be performed continuously. All reagents should be prepared before starting the assay procedure.

Literature

[1] Behrman, A; Buchta, WG; Budnick, LD; Hodgson, MJ; Raymond, LW; Russi, M; Spillmann, SJ; Swift, MD (Aug 2013). "Protecting Health Care Workers From Tuberculosis, 2013: ACOEM Medical Center Occupational Health Section Task Force on Tuberculosis and Health Care Workers.". Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine 55 (8): 985–8. doi:10.1097/JOM.0b013e3182a0d7cd. PMID 23887706.

[2] Bellete, B; Coberly, J; Barnes, GL; Ko, C; Chaisson, RE; Comstock, GW; Bishai, WR (Jun 1, 2002). "Evaluation of a whole-blood interferon-gamma release assay for the detection of *Mycobacterium tuberculosis* infection in 2 study populations.". Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 34 (11): 1449–56. doi:10.1086/340397. PMID 12015690.

[3] CDC, Centers for Disease Control and Prevention CDC 24/7: Saving Lives, Protecting People: Updated Guidelines for Using Interferon Gamma Release Assays to Detect *Mycobacterium tuberculosis* Infection, United States. MMWR 2010; 59 (No.RR-5): https://www.cdc.gov/tb/publications/factsheets/testing/igra.htm.

[4] Cecilia S. Lindestam Arlehamn,David Lewinsohn, Alessandro Sette, and Deborah Lewinsohn: Antigens for CD4 and CD8 T Cells in Tuberculosis. Cold Spring Harb Perspect Med. 2014 Jul; 4(7): a018465, 1-15. doi: 10.1101/cshperspect.a018465.

[5] "Cytokine" in John Lackie. A Dictionary of Biomedicine. Oxford University Press. 2010. ISBN 9780199549351.

[6] "Cytokine" in Stedman's Medical Dictionary, 28th ed. Wolters Kluwer Health, Lippincott, Williams & Wilkins (2006) http://en.wikipedia.org/wiki/Interferon_gamma.

[7] Diel, R; Loddenkemper, R; Meywald-Walter, K; Niemann, S; Nienhaus, A (May 15, 2008). "Predictive value of a whole blood IFN-gamma assay for the development of active tuberculosis disease after recent infection with *Mycobacterium tuberculosis*.". American Journal of Respiratory and Critical Care Medicine 177 (10): 1164–70. doi:10.1164/rccm.200711-1613OC. PMID 18276940.

[8] Elena Chiappini, Chiara Della Bella, Francesca Bonsignori, Sara Sollai, Amedeo Amedei, Luisa Galli, Elena Niccolai, Gianfranco Del Prete, Mahavir Singh, Mario M. D'Elios, and Maurizio de Martino: Potential Role of *M. tuberculosis* Specific IFN-γ and IL-2 ELISPOT Assays in Discriminating Children with Active or Latent Tuberculosis. PLoS One. 2012; 7(9): e46041. Published online Sep 28, 2012. doi: 10.1371/journal.pone.0046041

[9]"Entrez Gene: IFNGR2".

[10] Franken, WP; Timmermans, JF; Prins, C; Slootman, EJ; Dreverman, J; Bruins, H; van Dissel, JT; Arend, SM (Apr 2007). "Comparison of Mantoux and QuantiFERON TB Gold tests for diagnosis of latent tuberculosis infection in Army personnel.". Clinical and vaccine immunology : CVI 14 (4): 477–80. doi:10.1128/CVI.00463-06. PMC 1865601. PMID 17301213.

[11] Gerald H. Mazurek, M.D., Margarita E. Villarino, M.D. "Guidelines for Using the QuantiFERON-TB Test for Diagnosing Latent *Mycobacterium tuberculosis* Infection". Retrieved 2007-06-01.

[12] Gray PW, Goeddel DV (August 1982). "Structure of the human immune interferon gene". Nature 298 (5877): 859–63. doi:10.1038/298859a0. PMID 6180322.

[13] Green JA, Cooperband SR, Kibrick S (1969). "Immune specific induction of interferon production in cultures of human blood lymphocytes". Science 164 (3886): 1415–1417. doi:10.1126/science.164.3886.1415. PMID 5783715.

[14] Horst Ibelgaufts. Cytokines in Cytokines & Cells Online Pathfinder Encyclopedia Version 31.4 (Spring/Summer 2013 Edition).

[15] Mazurek, GH; LoBue, PA; Daley, CL; Bernardo, J; Lardizabal, AA; Bishai, WR; Iademarco, MF; Rothel, JS (Oct 10, 2001). "Comparison of a whole-blood interferon gamma assay with tuberculin skin testing for detecting latent *Mycobacterium tuberculosis* infection.". JAMA: the Journal of the American Medical Association 286 (14): 1740–7. doi:10.1001/jama.286.14.1740. PMID 11594899.

[16] Milstone, LM; Waksman BH (1970). "Release of virus inhibitor from tuberculin-sensitized peritoneal cells stimulated by antigen". J Immunol 105 (5): 1068–1071. PMID 4321289.

[17] Pottumarthy, S; Morris, AJ; Harrison, AC; Wells, VC (Oct 1999). "Evaluation of the tuberculin gamma interferon assay: potential to replace the Mantoux skin test.". Journal of clinical microbiology 37 (10): 3229–32. PMC 85534. PMID 10488182.

[18] Schoenborn JR, Wilson CB (2007). "Regulation of interferon-gamma during innate and adaptive immune responses". Adv. Immunol. 96: 41–101. doi:10.1016/S0065-2776(07)96002-2. PMID 17981204.

[19] Streeton, JA; Desem, N; Jones, SL (Jun 1998). "Sensitivity and specificity of a gamma interferon blood test for tuberculosis infection.". The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease 2 (6): 443–50. PMID 9626600.

[20] Thanassi, Wendy; Noda, Art; Hernandez, Beatriz; Newell, Jeffery; Terpeluk, Paul; Marder, David; Yesavage, Jerome A. (2012). "Delineating a Retesting Zone Using Receiver Operating Characteristic Analysis on Serial QuantiFERON Tuberculosis Test Results in US Healthcare Workers". Pulmonary Medicine 2012: 1–7. doi:10.1155/2012/291294.

[21] Wheelock, EF, Interferon-like virus inhibitor induced in human leukocytes by phytohemagglutinin. Science 149, 310-311, 1965. It was also shown to be produced in human lymphocytes.

[22] WHO (2018). "Latent TB Infection: Updated and consolidated guidelines for programmatic management". WHO/CDS/TB/2018.4, ISBN 978-92-4-155023-9, Page 1-74 (www.who.int/tb/publications/2018/latent-tuberculosis-infection/en/)

[23] Zwerling, A; Benedetti, A; Cojocariu, M; McIntosh, F; Pietrangelo, F; Behr, MA; Schwartzman, K; Menzies, D; Pai, M (2013). "Repeat IGRA testing in Canadian health workers: conversions or unexplained variability?". PLoS ONE 8 (1): e54748. doi:10.1371/journal.pone.0054748. PMID 23382955.

[24] Global tuberculosis report 2024; WHO http://www.who.int/tb/publications/global_report/en/

[25] Tamara Davenne and Helen McShane. Why don't we have an effective tuberculosis vaccine yet? Expert Rev Vaccines. 2016 Aug 2; 15(8): 1009–1013. Published online 2016 May 3. doi: 10.1586/14760584.2016.1170599 PMCID: PMC4950406. PMID: 27010255

[26] Indeterminate results of a tuberculosis-specific interferon-γ release assay in immunocompromised patients. Eur Respir J 2010; 35: 1179–1191. DOI: 10.1183/09031936.00122109.CopyrightERS Journals Ltd 2010.

[27] Chiara Della Bella, Michele Spinicci, Heba F. Mustafa Alnwaisri, Eduard Arkadievich Shuralev, Alessandro Bartoloni, Mario Milco D'Elios. LIOFeron®TB/LTBI: A novel and reliable test for LTBI and tuberculosis. December 23, 2019DOI: https://doi.org/10.1016/j.ijid.2019.12.012

[28] Della Bella, Chiara, et al. "Performance evaluation of the LIOFeron® TB/LTBI IGRA for screening of paediatric LTBI and tuberculosis." European Journal of Pediatrics 184.2 (2025): 147

Symbols

IVD	Only for in vitro diagnostic use
CE	Compliant with IVD Directive 98/79/EG
GTIN	Global Trade Item Number
REF	Catalogue number
LOT	Batch number
	Manufacturer
Σ	For <x> number of tests
	Expiry date

2°C 8°C	Store at 2 – 8 °C
2°C 30°C	Store at 2 – 30 °C
	Consult instructions for use
	Single use only
	Do not use if the outer packaging is damaged
	Protect from humidity
	Protect from direct light

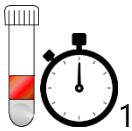
PC	Positive control
NC	Negative control
MTP	Mikrotiter plate
STA	Recombinant human IFN-γ standard
DET	Detection Antibody Solution
CON	Conjugate Solution

TB A	TB Antigen A
TB B	TB Antigen B
TMB	Substrate Solution
STO	Stop Solution
WB	Wash Buffer, 10x concentrate
IB	Incubation Buffer

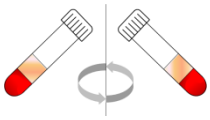
Abbreviated Test Procedure

Step 1 - HUMAN BLOOD STIMULATION TUBES

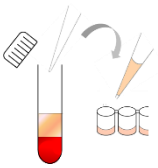
Collecting whole blood from the vein: Under standard laboratory conditions (aseptic, avoid haemolysis), collect at least **4.5 mL** human blood using a **lithium heparin** blood collection tube.



Gently invert the lithium heparin blood collection tube at least 10 times. Transfer 1 mL of the blood sample into each HUMAN BLOOD STIMULATION TUBE NC, TB A, TB B and PC and gently invert 10 times. Immediately place the HUMAN BLOOD STIMULATION TUBES upright into an incubator and incubate them for **16 - 24 hours lang at 37 °C (± 0.5 °C)**.



Harvest the human plasma by centrifuging the tubes for **15 minutes** at **2000 to 3000 RCF (g).** The red blood cells accumulate in the gel plug which separates the cells from the plasma.



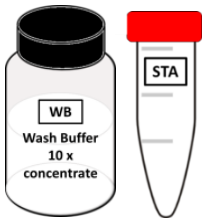
Transfer 50 µL of the clear supernatant (human plasma) into the wells of the microtiter plate and continue with step 5 the "HUMAN IFN-γ ELISA Test Procedure" (step 4 of the abbreviated HUMAN IFN-γ ELISA procedure). Optionally, the samples can be stored as described in section "*Use and Storage of Stimulated Plasma*".

Avoid contamination with red blood cells!

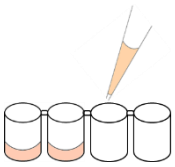
Step 2 - HUMAN IFN-γ ELISA

30 min

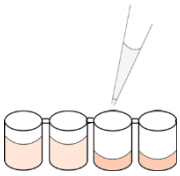
Bring all kit components to **room temperature** (15 – 30 °C).



Dilute **Wash Buffer (10x concentrate)** with distilled water to 1x Wash Buffer. Reconstitute The STA **recombinant human IFN-γ standard** to a 12.5 IU/mL stock solution by adding the indicated amount of IB Incubation Buffer and mix by vigorous shaking (e.g. vortexing for 10 seconds). Let the reconstituted standard rest for at least 5 minutes at room temperature. Prepare the other standard solutions **S1 - S4** (see section "*Preparation of Reagents*").



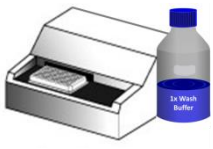
Pipette **50 µL** of each sample as well as the prepared STA standard solutions **S1 - S3** and the IB Incubation Buffer as **blank (S4)** into the wells of the MTP Microtiter plate. Stored plasma must be mixed shortly before use. The **standards and the blank** must be assayed **in duplicate**; samples can be measured as **single determination**.



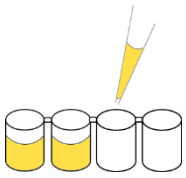
Pipette **50 µL** DET **Detection Antibody Solution** into each well of the MTP Microtiter plate. Mix the standards and samples thoroughly with a microtiter plate shaker for **60 seconds** at **500 to 1000 rpm**.

60 min

INCUBATION PERIOD: **1 hour (± 5 minutes) at 15 – 30 °C**

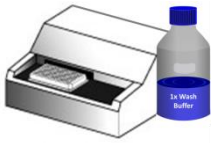


Wash the MTP Microtiter plate **6x** with **400 µL diluted (1x) Wash Buffer** per Well.

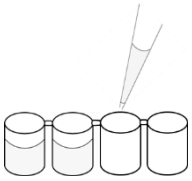


Pipette **100 µL** CON **Conjugate Solution** into the well of the MTP Microtiter plate.

INCUBATION PERIOD: **1 hour (± 5 minutes) at 15 – 30 °C**

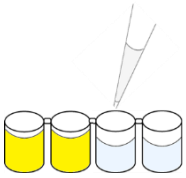


Wash the MTP Microtiter plate **6x** with **400 µL diluted (1x) Wash Buffer** per Well.



Pipette **100 µL** TMB **Substrate Solution** into each well of the MTP Microtiter plate.

INCUBATION PERIOD: **30 Minutes (± 1 minute) at 15 – 30 °C in the dark**



Quickly add **50 µL** of the STO **Stop Solution** to the TMB Substrate Solution into each well of the MTP Microtiter plate, to terminate the substrate reaction.

Do not wash!

Measure the absorption (OD) at **450 nm** (optional reference wavelength: 620 nm). The colour is stable for at least 60 minutes.

Analyse the results (software or manually by following the instructions for use).

Note: Please check www.lionex.de for specific software.

Trademarks:
LIONEX®, LIOFeron®, Microsoft®, Excel® (Microsoft), QuantiFERON® (Qiagen)
Limited License Agreement for LIOFeron® TB/LTBI - 2-component kit consisting of HUMAN BLOOD STIMULATION TUBES and HUMAN IFN-γ ELISA.

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used only with the instructions provided in the kit and the kit components. LIONEX GmbH grants no license for any of the contained components of this kit or any components which are not included within this kit (except as described in the instruction and the kit) or of intellectual property.

2. Other than expressly stated licenses, LIONEX GmbH makes no warranty that the kit and/or its use(s) do not infringe the rights of third parties.

3. The kit and its components are licensed for one-time use and may not be use, refurbished, or resold unless otherwise defined by LIONEX GmbH.

4. LIONEX GmbH especially disclaims any other licenses, remarked or signified other than those expressly specified.

5. The purchaser and user of the kit agree not to permit anyone else to take any steps which could lead to or facilitate any deed prohibited above. LIONEX GmbH may execute the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, attorney fees, in any action to execute this Limited License Agreement or any of the intellectual property rights which are relating to the kit and the kit components.

For updated license terms, visit www.lionex.de.