HUMAN BLOOD STIMULATION TUBES

Instructions for use

Interferon Gamma (IFN-γ) Release Assay (IGRA) in human blood for diagnosing Latent TB Infection (LTBI). As for other IGRA tests on the market, this test may react positive in TB patients also, but it cannot differentiate between LTBI and active TB. For professional in vitro diagnostic use only! Not for personal use!

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Intended use

The **LIOFeron®TB/LTBI** is a **2 component kit** consisting of **HUMAN BLOOD STIMULATION TUBES (component 01)** and **HUMAN IFN-γ ELISA (component 02)**. It is a cytokine release assay for quantitative determination of Interferon Gamma (IFN-γ) produced by human blood cells stimulated with *Mycobacterium tuberculosis* antigens. Thus, the test is useful for the diagnosis of Latent TB Infection (LTBI). As for other IGRA tests on the market, this test may react positive in TB patients also, but it cannot differentiate between LTBI and active TB. The test is intended for professional in vitro diagnostic use. The test is NOT intended for personal use.

Important note: HUMAN BLOOD STIMULATION TUBES cannot be used without the 2nd component **HUMAN IFN-γ ELISA** (Catalog no. LIO-Feron 02_22 or LIO-Feron 02_44). The **HUMAN IFN-γ ELISA** can be ordered from our sales department. The kit contains detailed instructions for use of **LIOFeron®TB/LTBI - 2 Component Kit**. The detailed instructions can be supplied by our sales department separately. Please contact us by e-mail, fax or postal mail (refer to manufactures identification above or contact information on the last page).

Principles of the test

The **LIOFeron®TB/LTBI** is a cytokine release assay based on the fact that cells from human blood will secrete IFN-γ when exposed to special *M. tuberculosis* antigens. The **HUMAN BLOOD STIMULATION TUBES** contain a positive control tube, a negative control tube and TB antigen tubes for each patient. The human blood sample (heparinized) is taken from the patient by venipuncture and 1 mL each is pipetted into negative-, positive- and TB antigen tubes. The tubes are gently mixed by shaking upside down and placed into a 37°C incubator overnight. Next the clear supernatant (human plasma) is carefully removed and analyzed using **HUMAN IFN-γ ELISA** which quantitates the amount of IFN-γ produced in response to the antigens from *M. tuberculosis*. These special antigens are distinguishable from those present in BCG and most other non-tuberculous mycobacteria. For detailed description of **HUMAN IFN-γ ELISA** which is based on the principle of the enzyme immunoassay (EIA) refer to the corresponding instructions for use.

Supplied Materials - Component 01

HUMAN BLOOD STIMULATION TUBES:					
Cap colour		Σ REF	1 Test **LIO-Feron 01_1**	22 Tests **LIO-Feron 01_22**	2x 22 Tests **LIO-Feron 01_22** (2x)
■	Positive control (ready-to-use) contains Li-Heparin and mitogen (black cap)	PC	1 tube	22 tubes	2x 22 tubes
□	Negative control (ready-to-use) contains Li-Heparin (white cap)	NC	1 tube	22 tubes	2x 22 tubes
■	TB antigen A (ready-to-use) contains Li-Heparin and LIONEX antigen missing in BCG (green cap)	TB A	1 tube	22 tubes	2x 22 tubes
■	TB antigen B (ready-to-use) contains Li-Heparin and LIONEX antigen with CD8+ epitope (blue cap)	TB B	1 tube	22 tubes	2x 22 tubes

Materials needed but not provided

- Disposable gloves; Waste containers for potentially contaminated materials
- Li-Heparin blood collection tubes
- Tubes for plasma collection and dilution (optionally; e.g. sterile 1.5 mL tube)
- Incubator 37°C (± 0.5 °C), CO_2 not essential
- Centrifuge suitable for blood tubes (optionally, RCF range from 2000 to 3000)

Preparation of reagents

The **HUMAN BLOOD STIMULATION TUBES** are ready-to-use, no prepartion of reagents is required.

Stability and storage conditions

Store at 2 - 30°C. DO NOT EXPOSE the test components to temperatures above 30°C. Unopened kit components are stable until the expiry date. The expiry date is printed on the labels of each test component and on the outer packaging.

Warnings and Precautions

Follow the instructions of the test procedure carefully! In accordance with Good Laboratory Practice (GLP), all laboratory devices employed should be regularly checked and calibrated for the accuracy and precision. Do not ingest or swallow! Do not eat, drink and smoke in the laboratory! Do not work without wearing protective clothing (disposable gloves, safety glasses and lab coat)! Use all reagents within the expiry period (printed on the labels). Bring all test components to room temperature (preferably 15 - 30°C). The test is sensitive to temperatures above 30°C. Use only fresh blood samples containing anticoagulants (Li-Heparin). Do not use body fluids other than Li-Heparin human blood because other are not validated or can yield incorrect results (e.g. citrate blood inhibits assay performance). Store blood samples at room temperature (preferably 15 - 30°C)! Do not store blood samples below 15°C! Do not freeze the blood sample. The vitality of cells can not been guaranteed after incorrect or prolonged blood storage. The blood shall not be used, if it is older than 16 hours after venipuncture. Do not use reagents from different kit lots or batch codes and do not mix reagents of different kit lots or batch codes. Work under sterile conditions to avoid contamination of samples. While collecting the supernatant avoid contamination by red blood cells. If necessary, separate the red blood cells from plasma by centrifugation. Avoid touching of the inlet of vial screw cap with your fingers (danger of contamination). For single-use only. Do not use if the vials are damaged or open (no screw cap). For more information, please request the **Material Safety Data Sheets (MSDS)** via E-mail to sales@lionex.de.

ATTENTION:
Handle human blood and plasma as potential infectious. All kit components should be considered as infectious agents. Decontaminate and dispose remaining kit reagents and human blood samples in accordance with federal, state and local regulations, e.g. by autoclaving or using a disinfecting solution.

Sample collection and preparation

The **LIOFeron®TB/LTBI** works best with fresh human blood samples.
Minimum 4.5 mL human blood collected under standard laboratory conditions (aseptically, avoid haemolysis) by using Li-Heparin blood collection tube is needed.
If the application of **HUMAN BLOOD STIMULATION TUBES** cannot be performed immediately after blood sampling from the vein, the whole blood can be stored for up to 16 hours at 15 - 30°C.

Use and storage of stimulated plasma

We recommend to transfer the plasma directly after centrifugation from the tubes to the ELISA plate. Hence, we suggest to perform the HUMAN IFN-γ ELISA within a few days after blood stimulation.
If the HUMAN IFN-γ ELISA test cannot be performed immediately after stimulation, the tubes can be stored for up to 4 days at 2 – 8°C.
For longer storage separate human plasma from the red blood cells and store at 2 - 8°C (up to 28 days). But this may lead to decrease in the concentration of IFN-γ. If harvested, plasma samples can be stored for extended periods below -20°C. Frozen samples must be thawed prior to testing and well mixed. Avoid repeated freezing and thawing of samples!

Test procedure

HUMAN BLOOD STIMULATION TUBES

Test procedure time requires 10 minutes. Incubation time: 16 - 24 h.

1. Take the required number of **HUMAN BLOOD STIMULATION TUBES** NC, TB A, TB B and PC from the kit. Place them in a rack/holder. Label the **HUMAN BLOOD STIMULATION TUBES** appropriately, e.g. by sample number or ID. Remove the lid of each tube by **pulling up the cap (no screwing)** and place them on flat surface.

2. Invert the Li-Heparin blood collection tube several times upside down. Take care that the sample is homogeneous. If a pellet is visible when the tube is turned over, loosen it carefully (e.g. by gently shaking). Transfer 1 mL of the blood sample into each **HUMAN BLOOD STIMULATION TUBE** for each individual (NC, TB A, TB B and PC).

3. Mix each **HUMAN BLOOD STIMULATION TUBES** filled with 1 mL Li-Heparin blood 10 x gently upside down. Avoid vigorous shaking; otherwise the blood cells could hemolyze!

4. Immediately place the rack with **HUMAN BLOOD STIMULATION TUBES** filled with 1 mL Li-Heparin blood upright in an incubator at 37°C (± 0.5 °C) for minimum 16 hours. Maximum incubation duration is 24 hours.

5. Finally, take out the rack with **HUMAN BLOOD STIMULATION TUBES** from the 37°C incubator (without shaking!). **It is possible to harvest the plasma without centrifugation but take care to avoid contamination with red blood cells**. The red blood cells accumulate in the gel plug which separates the cells from the plasma.

> **Important note: If contamination with red blood cells is observed** harvest the human plasma by centrifugation of the tubes for 15 minutes at 2000 to 3000 RCF (g). After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times take care not to disturb material on the surface of the gel.

6. Plasma samples should only be harvested using a pipet. Load directly from **HUMAN BLOOD STIMULATION TUBES** into the **HUMAN IFN-γ ELISA** plate. Transfer 50 µL of the clear supernatant (human plasma) from the HUMAN BLOOD STIMULATION TUBES into wells of microtiter plate and continue with step 5 of "HUMAN IFN-γ ELISA Test procedure".

Note: It is possible to use an automated ELISA workstation.

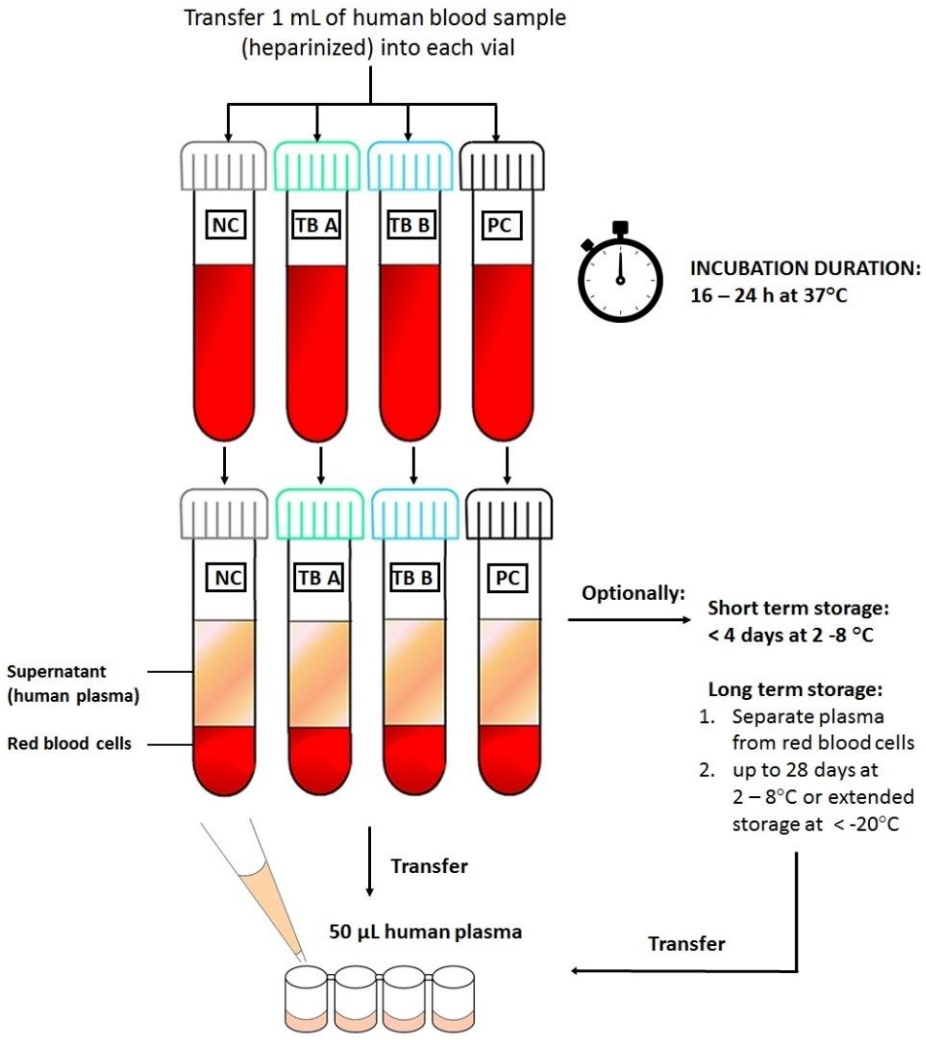
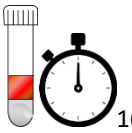


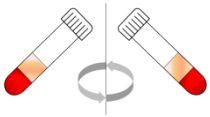
Figure 1:
Quick Reference Guide for using **HUMAN BLOOD STIMULATION TUBES**. Transfer the samples (supernatant) by directly pipetting into the ELISA plate. It is also possible to use automatic ELISA workstation. Optionally store at 2 – 8°C or below -20°C as indicated in "Use and storage of stimulated plasma".

Abbreviated Test procedure

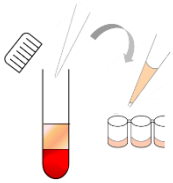
Collection of whole blood from the vein: Take minimum **4.5 mL** human blood under standard laboratory conditions (aseptically, avoid haemolysis) by using **Li-Heparin** blood collection tube.



Invert Li-Heparin blood collection tube approx. 3 x gently upside down. Transfer **1 mL of the blood** sample into each **HUMAN BLOOD STIMULATION TUBE** NC, TB A, TB B and PC and **mix 10 x gently upside down**. Immediately place the **HUMAN BLOOD STIMULATION TUBES** upright in an incubator at **37°C (± 0.5 °C) for 16 - 24 hours**.



Harvest the human plasma by **centrifugation** of 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) directly into wells of microtiter plate and continue with step 5 of "HUMAN IFN-γ ELISA Test procedure" in instructions for use of HUMAN IFN-γ ELISA Kit. Optionally transfer plasma samples to fresh labelled tubes (e.g. 1.5 mL tubes) and for maximum 28 days at 2 - 8°C before IFN-γ determination. **Avoid contamination by red blood cells!**

Calculation and Test Interpretation

HUMAN BLOOD STIMULATION TUBES cannot be used without the 2nd component **HUMAN IFN-γ ELISA** and does not produce any test result on its own. For ordering **HUMAN IFN-γ ELISA** or detailed instructions of **LIOFeron®TB/LTBI** please contact our sales department.

Quality control of test

HUMAN BLOOD STIMULATION TUBES cannot be used without the 2nd component **HUMAN IFN-γ ELISA**. For quality control instructions please refer to detailed instructions for use of **LIOFeron®TB/LTBI**. Please contact our sales department.

Limitations

Use only fresh blood samples containing anticoagulants (Li-Heparin). Do not use body fluids other than Li-Heparin human blood because other are not validated or can yield incorrect results (e.g. citrate blood inhibits assay performance). Do not use haemolysed or lipaemic samples. **HUMAN BLOOD STIMULATION TUBES** are intended to be used in combination with **HUMAN IFN-γ ELISA and cannot produce a test result on its own**. For further limitations of **LIOFeron®TB/LTBI** refer to detailed instructions for use. Please contact our sales department.

Performance Characteristics

HUMAN BLOOD STIMULATION TUBES cannot be used without the 2nd component **HUMAN IFN-γ ELISA**. For information on assay performance please refer to detailed instructions for use of **LIOFeron®TB/LTBI**. Please contact our sales department.

References

HUMAN BLOOD STIMULATION TUBES cannot be used without the 2nd component **HUMAN IFN-γ ELISA** and does not produce any test result alone. For detailed instructions of **LIOFeron®TB/LTBI** including references please contact our sales department.

Symbols

CE	Compliant with IVD Directive 98/79/EG		Manufacturer		Consumables: use by … (expiry date)
REF	Catalogue Number	Σ	Contains enough for <n> tests		Please consult instructions for use
LOT	Batch code		Do not reuse		Do not use if damaged
PC	Positive control	IVD	For in vitro diagnostic use	2°C 30°C	Store at 2 - 30°C (temperature limitation)
TB B	TB antigen B	NC	Negative control	TB A	TB antigen A