lophius biosciences

T CELL COMPANY

T-TRACK® human IFN-γ

INSTRUCTIONS FOR USE

The T-Track® ELISpot kit human IFN-γ HiSpecificity^{PRO} is a highly specific immunoassay based on the ELISpot technology. It is designed to analyse human IFN-γ secreting cells at cellular level.

FOR RESEARCH USE ONLY

REF 12200002 (solid plate)
12200001 (strip plate)

Manufacturer
Lophius Biosciences GmbH
Am Biopark 13
D-93053 Regensburg, Germany

TABLE OF CONTENTS

COMPONENTS OF THE T-TRACK® KIT

	Component		Unit	Amount	
1		Ready-MTP IFN-γ	Quantity	1	Microtiter-plate[1]
2	K50073	PHA[2]	Volume	70 µl	Concentrate
3	K50005	mAb-AP	Volume	70 µl	Concentrate
4	K50006	DB	Volume	12 ml	ready to use
5	K50007	Stain	Volume	6 ml	ready to use
6	K50048	WB1	Volume	200 ml	ready to use
7	K50049	WB2	Volume	70 ml	ready to use
8	K50026	Instructions for use	Quantity	1	

1) Type of plate (strips or solid 96 depends on kit (see label)
2) Positive control CONTROL +

STORAGE AND STABILITY

Storage

Store all components of the kit at 2 - 8 °C. The Stain solution 5 is light sensitve and has to be stored protected from light. Unused test strips must be replaced in the original packaging under sterile working conditions and protected from mechanical damage. Unused test strips and opened components have to be stored at 2 – 8 °C until further use.

Stability

The components of the kit are stable up to the expiry date (see front of box) when stored under the recommended conditions. Components used repeatedly have to be stored at 2 – 8 °C until further usage and are stable for up to 3 months after initial opening of the kit, at most up to the expiry date.

ADDITIONAL EQUIPMENT AND MATERIAL REQUIRED

- Sterile pipettes and pipette tips
- Sterile Pasteur pipettes
- Sterile cell culture medium (recommended: AIM-V®, Invitrogen)
- Tap water
- Neubauer counting chamber with microscope or validated device for cell counting
- Trypan Blue (optional)
- Class II microbiological cabinet
- Humidified incubator (37 °C, 5% CO_2)
- ELISpot reader (recommended)

PRINCIPLE OF THE T-TRACK® KIT

The T-Track® ELISpot kit human IFN-γ HiSpecificity^{PRO} assay is based on the highly sensitive ELISpot technique (Enzyme Linked Immunosorbent Spot). This method enables a highly specific and sensitive detection of specific reactivated effector cells.
In principle, the ELISpot method is a solid-phase ELISA.
The cytokine secreted by reactive cells, is captured by a specific antibody immobilized on the membrane. A second enzyme-conjugated antibody against the cytokine (detection antibody) marks the bound cytokine. A soluble substrate is added to each well. By the enzymatic conversion of the soluble substrate to an insoluble precipitate, spots are generated on the membrane, representing footprints of antigen-reactive, cytokine producing cells. The spots can be detected and counted either with a microscope or with an automatic imaging system (ELISpot reader).

PREPARATIONS

Reagents
Set up working solutions:

	dilution factor	diluent
PHA ❷	1:10	medium (AIM-V©)
mAb-AP ❸	1:180	dilution buffer ❹

Samples
Count the living cells in a Neubauer counting chamber under the microscope (Trypan blue staining) or with a validated device for cell counting. Adjust counted cells to 2 x 10^6 PBMCs/ml AIM-V© medium (corresponds to 2 x 10^5 PBMCs per well). Exact amounts for other cell populations have to be determined individually.

Stimulants
Dilute your stimulants (proteins, peptides, peptidpools, lysates…) with cell medium (we recommend: AIM-V©) to adjust optimal working concentration (to be determined individually). For stimulation a volume of 50 µl per well is recommended. A broad selection of stimulants is available from Lophius Biosciences GmbH (www.Lophius.com).

Positive control (for T cells and PBMCs only)
Use the PHA ❷ working solution for stimulation of the same cell number as in your samples (e.g. 2 x 10^5 cells per well).

Negative control
Use culture medium with the same amount of unstimulated cells (or treated with non-stimulating peptides/antigens) as stimulated cells in your samples (2 x 10^5 cells per well).

PROCEDURE

Set up of test strips/test plate

1 Remove the microtiter plate (Ready MTP 1) from the packaging (under sterile conditions). ☐

For using test strips only; otherwise proceed at 4. ☐

2 Remove the redundant test strips from the frame of the Ready-MTP 1. ☐

3 Put back strips that will not be used into the aluminium pouch (to prevent damage) and reseal safely. ☐

4 Determine a pipetting scheme for your samples, negative- and positive control. ☐

Stimulation of the cells

1 Distribute the working solutions in the wells in accordance with your pipetting scheme:
- negative control: 50 µl (e.g. medium) per well ☐
- positive control: 50 µl PHA-working solution per well ☐
- your stimulants: 50 µl working solution per well ☐

2 Mix the adjusted cell suspension (see page 5: Preparations) directly before use. ☐

3 Add carefully 100 µl of cell suspension to each well.
Note: Change pipette tip after each well to avoid cross-contamination. ☐

4 Cover Ready-MTP 1 with the lid. ☐

5 Incubate the Ready-MTP 1 at 37 °C, 5 % CO_2 incubator (recomended for PBMCs 16 to 20 hours). ☐
Note: Incubation times can vary depending on the antigen and frequency of cytokine producing cells and therefore should be optimized by the testing laboratory. For comparability reasons within one experiment incubation time should be equal.

Detection of cytokine producing effector cells

1 Discard cell suspension and medium. ☐
2 Add 200 µl WB1 (6) per well. ☐
3 Discard the buffer and repeat the washing step 5 more times. ☐
Note: The procedure can be interrupted at this step. Remove the buffer from the last washing step and store the plate at -20°C for up to 48 h.
4 Add 100 µl mAb-AP working solution per well. ☐
5 Incubate the covered wells for 2 h at room temperature. ☐
6 Discard the mAb-AP working solution. ☐
7 Add 200 µl WB1 (6) per well. ☐
8 Discard the buffer and repeat the washing step 2 more times. ☐
9 Add 200 µl WB2 (7) per well. ☐
10 Discard the buffer and repeat the washing step 2 more times. ☐
11 Add 50 µl Stain (5) per well and cover the wells. ☐
12 Incubate the wells for 6 - 7 min at room temperature protected from light. ☐
Note: Avoid a longer incubation time as this may negatively effect the quantification of the results. ☐
13 Stop the staining reaction by washing the wells 3 times with tap water. ☐
14 Remove the bottom lid of the plate and wrap the plate in a paper towel. Strike the plate to remove residual droplets. ☐
15 Let the test strips dry over night at room temperature or by placing the plate in the air stream of a laminar flow cabinet for one hour. ☐
Note: After drying, the staining is stable for several weeks if protected from light.

READ OUT OF RESULTS

The developed spots are counted and the analysis, interpretation and documentation of the results are carried out according to the following instruction:

Quality control
The positive control (PHA 2) should show a strong response with a high spot count.

Results interpretation
Counted spots represent the signature of interleukin producing reactivated effector cells.

PERFORMANCE CHARACTERISTICS

The performance characteristics of T-Track® ELISpot kit human IFN-γ HiSpecificity^{PRO} were assessed using the Cytomegalovirus (CMV) proteins T-activated® IE-1 and T-activated® pp65 from Lophius Biosciences GmbH.

Measuring range and linearity
The assay performance characteristics and linearity depend on the measurement device used. To determine all assay performance characteristics the Bioreader® 5000 Eα was used.
In a range of 10 to 1,000 spots, the spot count is proportional to the amount of antigen reactive effector cells (e.g. PBMC). If more than 400 spots per well occur, a retesting of the sample with a dilution of 1:2 to max. 1:3 can be required, depending on the sensitivity of the used measuring device.
Per well, 6 x 10^4 to 2 x 10^5 PBMC are analyzable.

Repeatability
The repeatability was assessed from measurements of 3 test persons. From each test person, blood was taken for 3 independent measurements. The total of 9 blood samples were processed simultaneously and measured independently. The determined variation of the repeated measurements was less than 22 %.

Reproducibility
The assessment of the reproducibility was done similar to the repeatability. Again, blood was taken from 3 test persons for 2 independent measurements. For the measurements, only one of the following parameters was varied:
– Operator (3 different operators; all operators used the same amout of blood)
– Working conditions and environment (inter-site variation)
– Applied measurement devices (comparison of the different readers)

Reference
Barabas et al. (2008): Urea-mediated Cross-Presentation of Soluble Epstein-Barr Virus BZLF1 Protein. PLoS Pathogens 4 (11)

SAFETY WARNINGS AND PRECAUTIONS

Care should be taken when handling material of human origin. All blood samples should be considered potentially infectious. Handling, use, storage and disposal of blood samples and assay components should be according to national biohazard safety guidelines or regulations. Care should be taken when working with chemicals. All chemicals should be considered potentially hazardous. For further information please request the corresponding Material Safety Data Sheets (MSDS) via our website www.Lophius.com.

LIMITATIONS/REMARKS

- Read the assay instructions carefully before use.
- For professional and research use only.
- Store the kit at 2 - 8 °C. The kit must not be used beyond the expiry date.
- Do not mix components of different kit lots.
- Use sterile techniques to avoid contamination of the reagents, test strips, cell suspensions and cell culture media.
  Note: The expression "sterile technique" means a controlled working environment to avoid contaminations.
- Variations to the stated pipetting and washing techniques, incubation times and/or temperatures may influence the results. The recommendations in the instructions for use 8 must be observed.
- For the comparability of the test results, a validation of cell counting and measurement devices (concerning measuring range, measuring protocol) is recommended.
- Consider the limitations of the measurement devices.

NOTES

GLOSSARY OF SYMBOLS

	Sufficient for„n“ plate/strips
	Consult instructions for use
	Attention, see instructions for use
	Order number
	Lot number
	Use by/expiration date
	Temperature limitation/store between
	Positive control materials

Manufacturer
Lophius Biosciences GmbH
Am Biopark 13
93053 Regensburg, Germany
+49 941 63091970

K50026-EN
Issue 2016-06-07
Rev. 05.00