研究用試薬

Updates with respect to the previous version are marked in grey.

Quan-T-Cell ELISA

Instructions for use

For in vitro diagnostic use IVD

ORDER NO.	ANTIGEN	SUBSTRATE	FORMAT
EQ 6841-9601	Human interferon gamma	Ab-coated microplate wells	96 x 01 (96)

CE

Intended use

The Quan-T-Cell ELISA provides in vitro determination of interferon gamma in human heparinised plasma and may only be used in combination with a pathogen-specific stimulation tube set of the Quan-T-Cell product range. The respective intended use of the stimulation tube set must be observed. The product is designed for the use by healthcare professionals and can be processed and evaluated manually or on automated instruments. The results should always be interpreted together with those of other laboratory diagnostic procedures and based on the clinical picture.

Clinical significance

The cytokine interferon gamma is mainly formed by $CD4^+$ and $CD8^+$ T cells as well as natural killer cells. Interferon-gamma plays a central role in the protection from viruses and microorganisms. It activates macrophages and stimulates the specific cytotoxic immunity. Interferon gamma appears in early infection stages – prior to the antigen-specific adaptive immune response [1, 2].

Among other things, interferon gamma contributes to protection from infections with hepatitis B, Herpes simplex and lymphocytic choriomeningitis virus [2]. The immune response to cytomegalovirus (CMV) is also mainly cell-mediated – T cells produce interferon gamma to CMV [3]. Likewise, COVID-19 patients often present SARS-CoV-2-reactive interferon-gamma $CD8^+$ T cells [4]. Interferon-gamma-producing $CD4^+$ and $CD8^+$ T cells play the most important role in combatting human infections with *Mycobacterium tuberculosis* [5].

The Quan-T-Cell ELISA is an interferon-gamma-release assay (IGRA) providing determination of the cellular immune reaction of a patient to a pathogen. In a blood sample, the lymphocytes are stimulated with pathogen-specific antigens. Subsequently, the amount of released interferon gamma is measured. Currently, the IGRAs are mostly applied in diagnostics of CMV infections and tuberculosis [3].

Antibody

The reagent wells are coated with monoclonal anti-interferon-gamma antibody.

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Test principle

The test can only be performed using a corresponding stimulation tube set from the EUROIMMUN Quan-T-Cell product range, e.g. Quan-T-Cell SARS-CoV-2 (EUROIMMUN order no. ET 2606-3003).

The stimulation tube set consists of three stimulation tubes (e.g. BLANK, TUBE, STIM) for use with a whole-blood sample. From one whole-blood sample, three plasmas (BLANK, TUBE, STIM) are obtained after stimulation, which must strictly be analysed on the same Quan-T-Cell ELISA plate.

The calibrators and controls of the Quan-T-Cell ELISA must also be included on each ELISA plate.

The test kit contains microplate strips each with 8 break-off reagent wells coated with monoclonal anti-interferon-gamma antibody. In the first reaction step, undiluted calibrators and controls and plasma samples diluted in sample buffer are added to the coated reagent wells to bind interferon gamma. In two further reaction steps, a biotin-labelled anti-interferon-gamma antibody is added, which is enzymatically detected by means of a streptavidin-bound horseradish peroxidase. The resulting colour intensity is proportional to the concentration of interferon-gamma antigen in the samples.

Contents of the test kit

Component	Colour	Format	Symbol
1. **Microplate wells coated with antibodies** 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	-	12 x 8	STRIPS
2. **Calibrator 1**, lyophilised	light red to dark red	1 x 1.0 ml	CAL 1
3. **Calibrator 2**, lyophilised		1 x 1.0 ml	CAL 2
4. **Calibrator 3**, lyophilised		1 x 1.0 ml	CAL 3
5. **Calibrator 4**, lyophilised		1 x 1.0 ml	CAL 4
6. **Calibrator 5**, lyophilised		1 x 1.0 ml	CAL 5
7. **Calibrator 6**, lyophilised		1 x 1.0 ml	CAL 6
8. **Control 1**, lyophilised	green	1 x 1.0 ml	CONTROL 1
9. **Control 2**, lyophilised	blue	1 x 1.0 ml	CONTROL 2
10. **Biotin**, biotin-labelled anti-interferon-gamma antibody, ready for use	green	1 x 12 ml	BIOTIN
11. **Enzyme conjugate** peroxidase-labelled streptavidin, ready for use	blue	1 x 12 ml	CONJUGATE
12. **Sample buffer** ready for use	blue	1 x 100 ml	SAMPLE BUFFER
13. **Wash buffer** 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
14. **Chromogen/substrate solution** TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
15. **Stop solution** 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
16. **Quality control certificate**	-	1 protocol	-
17. **Instructions for use**	-	1 booklet	-

Additional materials and equipment (not supplied in the test kit)

- Stimulation tube set of the Quan-T-Cell product range, e.g. Quan-T-Cell SARS-CoV-2 (EUROIMMUN order no. ET 2606-3003)
- Automatic microplate washer: recommended. Washing of the microplates can also be carried out manually.
- Microplate reader: wavelength of 450 nm, reference wavelength range from 620 nm to 650 nm
- Calibrated pipettes
- Pipette tips
- Stepper pipette: recommended for the pipetting of the sample buffer and biotin, conjugate, substrate and stop solutions
- Distilled or deionised water
- Incubator or water bath (+37 °C ± 1 °C): recommended to warm the wash buffer
- Stop watch
- Centrifuge with insert for 2-ml polypropylene tubes (6000 – 12,000 x g)
- Polypropylene tubes for predilution of the samples; not required with automated processing.

Storage and stability

The test kit has to be stored at a temperature between +2 °C and +8 °C; do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

In-use stability

After initial opening, reconstituted calibrators and controls are stable for only three months at -18 °C to -25 °C, meaning that an opened test kit must be used within this time frame. This stability is only given when all other kit components are stored at +2 °C to +8 °C and protected from contamination.

Warnings and precautions

- The product must only be used by healthcare professionals in an adequate laboratory environment.
- Do not use the test kit if the packaging of the reagents is damaged.
- Before using the product, read the instructions for use carefully. Only use the valid version provided with the product.
- EUROIMMUN reagents must not be mixed with or replaced by reagents from other manufacturers.
- Sample and wash buffers, substrate and stop solutions with identical article numbers (see label) are exchangeable independent of the lot. All other reagents are lot-specific and must not be combined with those from other lots.
- Observe Good Laboratory Practice (GLP) and safety guidelines. Some of the reagents contain preservatives in non-declarable concentrations. Avoid eye and skin contact with samples and reagents. In case of eye or skin contact, rinse thoroughly with water. Remove and wash contaminated clothing. In case of ingestion, obtain medical advice.
- The materials of human origin have tested negative for HBsAg, HIV antibodies and HCV RNA. Nonetheless, all test kit components should be treated as potentially infectious and handled with care.

Preparation and stability of the samples

Sample material

Human heparinised plasma obtained after stimulation with the EUROIMMUN stimulation tube set of the Quan-T-Cell product range (e.g. Quan-T-Cell SARS-CoV-2) (EUROIMMUN order no. ET 2606-3003)

Notes on sample handling

Plasma should be stored in polypropylene tubes (recommended screw-cap microtubes 2 ml, Sarstedt, item no. 72.609). When heparinised plasma is obtained from whole blood, contamination with cell components must be avoided.

Stability of the samples

Plasma samples in polypropylene tubes can be stored for up to 28 days at +2 °C to +8 °C. With longer storage, the samples should be frozen at -18 °C to -25 °C. Plasma samples are stable for 3 months at -18 °C to -25 °C and can be thawed maximally 4 times. Nonetheless, repeated freezing and thawing should be avoided.

Diluted samples must be incubated within a working day. They must not be used further but should be discarded.

Sample preparation

Bring sample buffer to room temperature (+18 °C to +25 °C) before use.

For ELISA measurement performed immediately after stimulation using the stimulation tube set of the EUROIMMUN Quan-T-Cell product range: Preparation of whole-blood samples

Remove the stimulated whole-blood samples contained in the EUROIMMUN stimulation tubes from the incubator and centrifuge for 10 minutes at 6000 – 12,000 x g prior to the ELISA measurement.
From this step on, make sure that the plasma is clearly separated from the cruor! Otherwise, the whole-blood sample must be centrifuged again.

Attention: Stimulated and centrifuged whole-blood samples must be analysed with the EUROIMMUN Quan-T-Cell ELISA within 24 h. Intermediate storage must take place at +2 to +8 °C for a maximum of 24 h. As the plasma must be separated clearly from the cruor EUROIMMUN recommends an additional centrifugation step after the cooled intermediate storage and before performing the ELISA measurement.

Automated processing of stimulated whole blood:

Following centrifugation, the stimulated whole-blood samples contained in the stimulation tubes can be loaded directly into the EUROIMMUN analysis instruments (EUROIMMUN Analyzer I, EUROIMMUN Analyzer I-2P and EUROLabWorkstation ELISA). For further information, please refer to the section "Fully automated test processing using analysis instruments".

Manual processing of stimulated whole blood:

Following centrifugation, the plasma obtained from the stimulated whole-blood samples is diluted. The sample buffer must be pipetted first. The plasma must be carefully aspirated off from above.

Example: For a 1:5 dilution, add 25 µl sample to 100 µl sample buffer and mix well (vortex).

Sample pipettes are not suitable for mixing.

Note: Reconstituted calibrators and controls are ready for use.

For ELISA measurement that is not performed immediately after stimulation using the stimulation tube set of the EUROIMMUN Quant-T-Cell product range: Preparation of stored stimulated plasma samples

After storage, bring the stimulated plasma samples to room temperature (+18 °C to +25 °C), mix thoroughly (vortex) and centrifuge for 10 minutes at 6000 to 12,000 x g.

Note: If the samples were frozen for storage, fibrin clots may have formed that can clog the pipette tip. Especially with automated processing, this may lead to false negative results. Frozen plasma samples may therefore not be treated without centrifugation and only be aspirated off from above.

Automated processing of stimulated plasma samples:

Following centrifugation, the plasma samples can be loaded into the EUROIMMUN analysis instruments (EUROIMMUN Analyzer I, EUROIMMUN Analyzer I-2P and EUROLabWorkstation ELISA). For further information, please refer to the section "Fully automated test processing using analysis instruments".

Manual processing of stimulated plasma samples:
Following centrifugation, the plasma samples to be investigated are diluted with sample buffer. The sample buffer must be pipetted first. The plasma must be carefully aspirated off from above.

Example: For a 1:5 dilution, add 25 µl sample to 100 µl sample buffer and mix well (vortex).

Sample pipettes are not suitable for mixing.

Note: Reconstituted calibrators and controls are ready for use.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18 °C to +25 °C) before use.

Sample buffer

Ready for use. Mix the reagent thoroughly before use.

Coated wells

Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
Once the protective wrapping has been opened for the first time, the wells coated with antibodies can be stored in a dry place and at a temperature between +2 °C and +8 °C for 4 months.

Calibrators and controls

Reconstitute calibrators and controls with 1 ml deionised or distilled water approximately 10 minutes before use, invert them and mix thoroughly upside down. Using a **vortexer** is highly recommended. Avoid foam formation in the calibrators and controls. Prior to use, make sure that the lyophilisate has completely dissolved in the water. If required, centrifuge the sample shortly in order to bring remaining liquid from the cap into the tube. Freeze the reconstituted calibrators and controls at -18 °C to -25 °C directly after use and avoid longer residence times at room temperature. When reusing reconstituted calibrators and controls, these must be slowly brought to room temperature (+18 °C to +25 °C) prior to use (warm for at least 30 minutes at room temperature) and mixed thoroughly (avoid foaming). Reconstituted calibrators and controls are stable for up to three months at -18 °C to -25 °C. They can be frozen and thawed up to six times.

Biotin

Ready for use. Mix the biotin thoroughly before use.

Enzyme conjugate

Ready for use. Mix the reagent thoroughly before use.

Wash buffer

The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37 °C ± 1 °C and mix well before dilution. Remove the required volume with a clean pipette tip and dilute 1:10 with deionised or distilled water (1 part reagent plus 9 parts water).

For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
The working-strength wash buffer is stable for 4 weeks if stored at +2 °C to +8 °C and handled properly.

Chromogen/substrate solution

Ready for use. Close the tube immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

Stop solution

Ready for use.

Waste disposal

Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Quality control

For every test performed, the extinction readings of the calibrators and the values determined for the controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing

the respective values is included. If the values specified for the calibrators and the controls are not achieved, the test results may be inaccurate and the test should be repeated.

Reference material

The calibrator material is adjusted to an international reference material (National Institute for Biological Standards and Control (NIBSC), 82/587, Non-WHO Reference Material). The quantification is made in milli-international units per millilitre (mIU/ml).

Assay procedure

(Partly) manual test performance

For performance of a quantitative test, the calibrators 1 to 6, the controls 1 and 2 and the patient samples are used.

Sample incubation: (1st step)
Transfer **100 µl** of the **calibrators, controls and diluted plasma samples (1:5 in sample buffer)** into the individual microplate wells according to the pipetting protocol. Cover the wells and incubate for **120 minutes** at room temperature (+18 °C to +25 °C).

Washing:
Manual: Empty the wells and subsequently wash **5 times using 300 µl of working-strength wash buffer** for each wash.
Automatic: Wash the reagent wells **5 times with 450 µl of working-strength wash buffer** (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note:
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Biotin incubation: (2nd step)
Pipette **100 µl of biotin** into each of the microplate wells. Cover the wells and incubate for **30 minutes** at room temperature (+18 °C to +25 °C).

Washing:
Empty the wells. Wash as described above.

Conjugate incubation: (3rd step)
Pipette **100 µl of enzyme conjugate** into each of the microplate wells. Cover the wells and incubate for **30 minutes** at room temperature (+18 °C to +25 °C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (4th step)
Pipette **100 µl of chromogen/substrate solution** into each of the microplate wells. Cover the wells and incubate for **20 minutes** at room temperature (+18 °C to +25 °C) protected from direct sunlight.

Stopping:
Pipette **100 µl of stop solution** into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution

Fully automated test processing using analysis instruments

Sample dilution and subsequent test performance can be carried out fully automatically using an analysis instrument. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the instructions for use of the ELISA. However, these conditions were validated in combination with the EUROIMMUN Analyzer I, the EUROIMMUN Analyzer I-2P, the EUROLabWorkstation ELISA and this EUROIMMUN ELISA.
Validation documents are available on enquiry.

- For automated processing in combination with the **EUROIMMUN Analyzer I** and **EUROIMMUN Analyzer I-2P**, the use of the software EUROLab Quan-T-Cell is recommended. For details, please refer to the respective instructions for use of the software, "Instructions for use EUROLab Quan-T-Cell" (EUROIMMUN file no. YG 6841_A_UK_CXX), and the respective short instructions.
    - This software supports the generation of barcodes for stimulation tubes of the EUROIMMUN Quan-T-Cell product range and performs the automated evaluation of the Quan-T-Cell ELISA (order no. EQ 6841-9601).
    - The loading into the EUROIMMUN Analyzer I/I-2P must be performed per patient to ensure that all three tubes of one stimulation tube set can be loaded one after another. The order of the three barcoded tubes BLANK, TUBE and STIM is not specifically predefined; however, it is recommended to maintain the initially chosen order for all patients to ensure smooth processing.
    - It is absolutely necessary that plasma samples of one stimulation tube set are incubated together on one plate according to the pipetting scheme (page 10). This ensures that the calculation of the concentrations of the respective conditions BLANK, STIM and TUBE of each whole-blood sample can be performed correctly. For further information, please refer to the instructions for use of the respective EUROIMMUN stimulation tube set of the Quan-T-Cell product range (e.g. Quan-T-Cell SARS-CoV-2, order no. ET 2606-3003, file no. ET_2606_A_UK_CXX).
    - A separate rack is required for the loading of calibrators and controls (Q-Rack). The loading of the samples contained in the stimulation tubes may require the set-up of new racks (Y-Rack). If other sample tubes are used according to the instrument specification, T-Racks must be used.

Note: When processing (on one of the EUROIMMUN Analyzers) without using the EUROLab Quan-T-Cell software, no automated test evaluation can be performed with the existing EUROIMMUN solutions. In addition, barcode generation for the stimulation tubes of the EUROIMMUN Quan-T-Cell series is not supported. Alternative software products for automated test evaluation as well as barcode generation support are not available from EUROIMMUN for the Analyzers.

- Automated processing on the **EUROLabWorkstation ELISA** is performed as described in the respective instructions for use.
    - The aliquotting barcodes of all three conditions of a whole-blood sample can be generated using a conventional barcode printing software.
    - The automated evaluation of samples obtained after stimulation is performed directly in the instrument software.
    - During loading into the EUROLabWorkstation ELISA, it is distinguished between:
        - Aliquots with material identifier, requiring no defined order for sample loading.
        - Aliquots without material identifier, requiring loading according to the manual pipetting scheme (page 10). With this variant, a maximum of 21 tubes may be loaded per rack.
        - For details, please refer to the respective short instructions of the EUROLabWorkstation ELISA.
    - Calibrators and controls are loaded via *Rack for Reagents in tubes*. For the loading of the biotin and the conjugate, an N-Rack must be additionally available. The sample buffer must be transferred into EUROTanks. The loading of the samples in the stimulation tubes may require the set-up of new racks (*Rack for reagents in tubes*). If other sample tubes are used according to the instrument specification, *Sample Racks* must be used.

Note: Processing on other fully automated systems is possible, but must be validated by the user.

Pipetting scheme

Note: The samples must be centrifuged prior to the loading (10 minutes at 6000 – 12,000 x g) and may not be mixed afterwards!

For the measurement using the EUROIMMUN Quan-T-Cell ELISA, the three plasma samples retrieved from one whole-blood sample must be pipetted following a defined scheme to prevent mix-up. The plasma samples of one blood sample must always be measured together on the same ELISA plate to enable correct subtraction of the interferon-gamma BLANK for the conditions STIM and TUBE.

A pipetting order which deviates from the instructions for use leads to incorrect calculation of the interferon-gamma concentration. Therefore, the below scheme must be strictly observed.

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 1	BLANK 1	STIM 3	TUBE 6	BLANK 9	STIM 11	TUBE 14	BLANK 17	STIM 19	TUBE 22	BLANK 25	STIM 27
B	C 2	TUBE 1	BLANK 4	STIM 6	TUBE 9	BLANK 12	STIM 14	TUBE 17	BLANK 20	STIM 22	TUBE 25	BLANK 28
C	C 3	STIM 1	TUBE 4	BLANK 7	STIM 9	TUBE 12	BLANK 15	STIM 17	TUBE 20	BLANK 23	STIM 25	TUBE 28
D	C 4	BLANK 2	STIM 4	TUBE 7	BLANK 10	STIM 12	TUBE 15	BLANK 18	STIM 20	TUBE 23	BLANK 26	STIM 28
E	C 5	TUBE 2	BLANK 5	STIM 7	TUBE 10	BLANK 13	STIM 15	TUBE 18	BLANK 21	STIM 23	TUBE 26	BLANK 29
F	C 6	STIM 2	TUBE 5	BLANK 8	STIM 10	TUBE 13	BLANK 16	STIM 18	TUBE 21	BLANK 24	STIM 26	TUBE 29
G	Co 1	BLANK 3	STIM 5	TUBE 8	BLANK 11	STIM 13	TUBE 16	BLANK 19	STIM 21	TUBE 24	BLANK 27	STIM 29
H	Co 2	TUBE 3	BLANK 6	STIM 8	TUBE 11	BLANK 14	STIM 16	TUBE 19	BLANK 22	STIM 24	TUBE 27	--

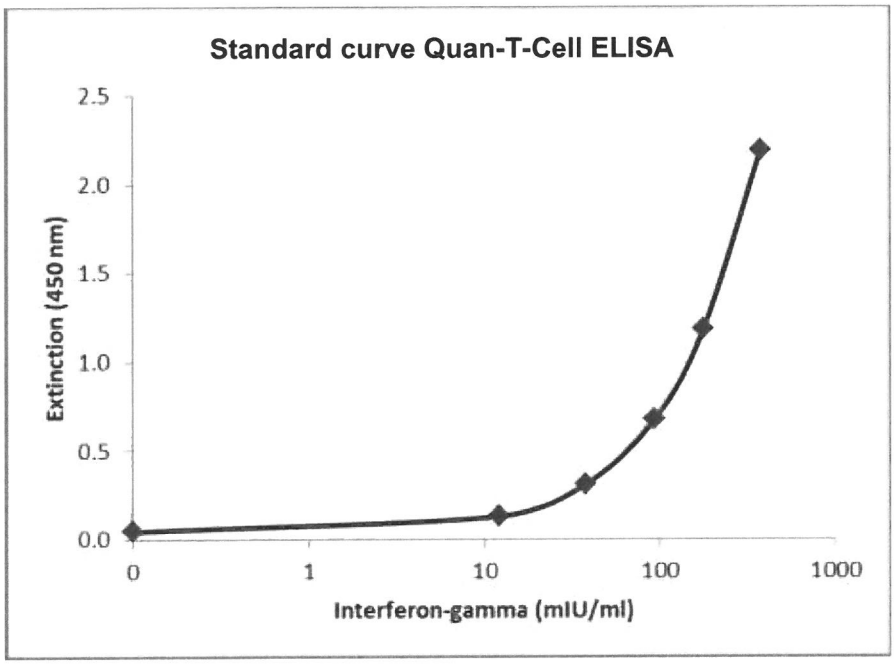
The pipetting scheme above applies to the quantitative analysis of plasma samples obtained using a EUROIMMUN stimulation tube set of the Quan-T-Cell product range (e.g. EUROIMMUN Quan-T-Cell SARS-CoV-2 order no. ET 2606-3003). The numbering from 1 to 29 refers to the whole-blood samples used for the test.

It is **absolutely necessary** that these plasma samples of one stimulation tube set are incubated together on one plate so that the calculation of the concentrations of the respective conditions BLANK, STIM and TUBE of each whole-blood sample can be performed correctly. For further information, please refer to the instructions for use of the respective EUROIMMUN stimulation tube set of the Quan-T-Cell product range (e.g. Quan-T-Cell SARS-CoV-2, order no. ET 2606-3003, file no. ET_2606_A_UK_CXX).
The controls 1 and 2 (Co 1 and Co 2) serve as internal controls for the reliability of the Quan-T-Cell ELISA measurement. They must be assayed with each test run.

Test evaluation

Quantitative: The standard curve from which the concentrations of the analyte in the samples can be taken is obtained by plotting of the extinction values measured for the six calibrators against the corresponding concentrations (linear/log). For computer-controlled calculation of the standard curve, the evaluation procedure "4-parameter Marquardt" or "cubic spline" should be selected. The standard dilution of 1:5 must be taken into account in the calculations by multiplying the values read from the standard curve by 5.

The following plot is an example of a typical calibration curve. Please do not use this curve to determine the analyte concentrations in the patient samples.



If the extinction of a sample lies above that of calibrator 6, it is recommended to further dilute this sample in sample buffer and measure it in a new test run according to the incubation instructions.

When preparing a dilution, first pipette the sample buffer into the polypropylene tubes and then add the sample.

Example of a 1:20 dilution: Pipette 190 µl sample buffer and add 10 µl sample

Example of a 1:10 dilution: Pipette 108 µl sample buffer and add 12 µl sample

This dilution must be taken into account in the subsequent calculation.

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

Qualitative evaluation:

For the evaluation of plasmas obtained using a EUROIMMUN stimulation tube set, please refer to the instructions for use of the respective Quan-T-Cell stimulation tube set of the EUROIMMUN Quan-T-Cell product range (e.g. Quan-T-Cell SARS-CoV-2 (ET 2606-3003), file no. ET_2606_A_UK_CXX).

Analytical performance

Lower measurement limits:

Limit of Blank (LoB): 8.76 mIU/ml
Limit of Detection (LoD): 18.44 mIU/ml
Limit of Quantitation (LoQ): 31.07 mIU/ml

Linearity

The linearity of the EUROIMMUN Quan-T-Cell ELISA was determined according to the CLSI guideline EP06-A. The EUROIMMUN Quan-T-Cell ELISA is linear at least in the investigated concentration range (79.10 mIU/ml to 1340.37 mIU/ml).

Intra-lab precision

Studies on the intra-lab precision were performed according to the CLSI guideline EP05-A3. Six samples (reactivity distributed over the entire measurement range) were measured. The precision is given as standard deviation (SD) and coefficient of variation (CV).

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
Mean	1158.32 mIU/ml		654.88 mIU/ml		355.35 mIU/ml		268.78 mIU/ml		134.38 mIU/ml		82.18 mIU/ml	
Components	*SD*	*% CV*	*SD*	*% CV*	*SD*	*% CV*	*SD*	*% CV*	*SD*	*% CV*	*SD*	*% CV*
Repeatability	26.813	2.3%	22.428	3.4%	10.222	2.9%	9.491	3.5%	6.350	4.7%	3.392	4.1%
Between-run	46.201	4.0%	12.867	2.0%	9.586	2.7%	5.813	2.2%	3.191	2.4%	3.263	4.0%
Within-day	53.417	4.6%	25.857	3.9%	14.014	3.9%	11.130	4.1%	7.107	5.3%	4.707	5.7%
Between-day	31.350	2.7%	17.503	2.7%	9.694	2.7%	9.279	3.5%	5.382	4.0%	3.938	4.8%
Within-lab	61.938	5.3%	31.224	4.8%	17.040	4.8%	14.491	5.4%	8.915	6.6%	6.137	7.5%

Reproducibility

Studies on the reproducibility were performed according to the CLSI guideline EP05-A3. Six samples (reactivity distributed over the entire measurement range) were measured. The reproducibility is given as standard deviation (SD) and coefficient of variation (CV).

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
Mean	1110.78 mIU/ml		631.55 mIU/ml		346.22 mIU/ml		258.60 mIU/ml		126.41 mIU/ml		79.45 mIU/ml	
Components	*SD*	*%CV*	*SD*	*% CV*	*SD*	*% CV*	*SD*	*% CV*	*SD*	*% CV*	*SD*	*% CV*
Repeatability	25.307	2.3%	16.958	2.7%	11.535	3.3%	8.641	3.3%	5.339	4.2%	3.704	4.7%
Between-run	21.273	1.9%	20.192	3.2%	9.235	2.7%	6.072	2.3%	4.304	3.4%	4.071	5.1%
Between-day	11.458	1.0%	0.000	0.0%	0.000	0.0%	2.115	0.8%	2.543	2.0%	2.497	3.1%
Within-lot	34.989	3.1%	26.368	4.2%	14.777	4.3%	10.770	4.2%	7.314	5.8%	6.044	7.6%
Between-lot	18.472	1.7%	0.000	0.0%	4.523	1.3%	2.749	1.1%	1.950	1.5%	1.315	1.7%
Reproducibility	39.566	3.6%	26.368	4.2%	15.453	4.5%	11.116	4.3%	7.569	6.0%	6.185	7.8%

Cross-reactivity (analytical specificity)

The Quan-T-Cell ELISA specifically detects interferon gamma. Significant undesired cross-reactions with other inflammatory cytokines (up to a concentration of 100 ng/ml each) that might affect the determination of interferon gamma in the patient samples were not observed.

Interleukin 2 2:	negative
Interferon-beta 1A:	negative
Interleukin 12:	negative
TNF-alpha:	negative
Interleukin 6:	negative
Interferon-alpha 2a:	negative

Interference

Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml haemoglobin, 20 mg/ml triglycerides and 0.4 mg/ml bilirubin in this ELISA. Contamination with fresh or frozen whole blood up to a percentage of 10% (v/v) and a biotin concentration of up to 10 µg/ml in the patient sample also had no influence on the results of this ELISA.

High-dose hook effect

A high-dose hook effect was neither observed in the native matrix (plasma), nor in the buffer up to a concentration of 100 IU/ml interferon gamma.

Limitations of the procedure

- The results should always be interpreted together with those of further laboratory diagnostic procedures and based on the clinical picture.
- A negative result in the Quan-T-Cell ELISA following stimulation with a Quan-T-Cell stimulation tube set of the EUROIMMUN Quan-T-Cell product range (e.g. Quan-T-Cell SARS-CoV-2 (ET 2606-3003)) does not exclude an infection or vaccination. Especially in an early infection phase or shortly after vaccination, the T cells have not yet or not sufficiently reacted to antigen stimulation. In the case of a borderline result, a secure evaluation is not possible. Collection and stimulation of a follow-up sample could lead to a clear result and is thus recommended. If there is a clinical suspicion and a negative or borderline serological result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. For this, plasma samples obtained from the BLANK condition may be used so that it is not necessary to take another sample. A positive result indicates a pathogen contact or vaccination.
- The specifications in the instructions for use, e.g. pipetting volumes, incubation times, temperatures and preparation steps must be observed to avoid incorrect results.
- Correct sample collection and storage are crucial for the reliability of the results.
- The interferon-gamma concentration can only be correctly determined if all plasma samples (BLANK, STIM, TUBE) of a stimulation tube set have been measured together on one plate.
- The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended to use a thermostatically adjusted ELISA incubator in all incubation steps.
- Insufficient washing (e.g. less than 5 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false extinction readings.
- Residual liquid (>10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction readings.
- Partial or complete adaptation of the test system for use with automated sample processors or other liquid handling devices may lead to differences between the results obtained with the automated and manual procedure. It is the responsibility of the user to validate the automated instruments used for the analysis to ensure that they yield test results within the permissible range.

Literature

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3. Albert-Vega C, Tawfik DM, Trouillet-Assant S, Vachot L, Mallet F, Textoris J. **Immune Functional Assays, From Custom to Standardized Tests for Precision Medicine.** Front Immunol. 2018; 9: 2367
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Liability

The test kit, including original accessories, must only be used in accordance with the intended use. EUROIMMUN accepts no liability for any other use (e.g. non-compliance with the instructions for use and improper use) or for resulting damages.

Technical support

In case of questions or technical issues, you can obtain assistance via the EUROIMMUN website (https://www.euroimmun.de/en/contact/).

You can find further information in the instructions for use of the EUROIMMUN stimulation tube set (e.g. EUROIMMUN SARS-CoV-2 IGRA stimulation tube set: order no. ET 2606-3003).

Additional information

Regulatory information for customers in the European Union: Please observe the obligation to report any serious incidents occurring in connection with this product to the competent authorities and to EUROIMMUN.

Meaning of the symbols

Symbol	Meaning	Symbol	Meaning
STRIPS	Microplate strips	CE	CE marking
CAL 1-6	Calibrators 1 - 6	SUBSTRATE	Substrate
CAL 1	Calibrator 1	STOP SOLUTION	Stop solution
CAL 2	Calibrator 2		Protect from sunlight
CAL 3	Calibrator 3	LOT	Lot description
CAL 4	Calibrator 4	REF	Order number
CAL 5	Calibrator 5	IVD	In vitro diagnostic medical device
CAL 6	Calibrator 6		Storage temperature
BIOTIN	Biotin		Unopened usable until (YYYY-MM-DD)
CONTROL 1	Control 1		Manufacturing date (YYYY-MM-DD)
CONTROL 2	Control 2		Manufacturer
CONJUGATE	Conjugate		Observe instructions for use
SAMPLE BUFFER	Sample buffer		Contents suffice for <n> analyses
WASH BUFFER 10x	Wash buffer, 10x concentrate		Biological risks