Interferon-gamma ELISA
Instruction for use

For research use only RUO

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

Antibody

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

Test principle

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

The stimulation tube set consists of three stimulation tubes (e.g. CoV-2 IGRA BLANK, CoV-2 IGRA TUBE, CoV-2 IGRA STIM) for use with a whole-blood sample. The analysis of the plasma obtained with the three stimulation tubes from one whole-blood sample must be performed on the same interferon-gamma ELISA plate. Additionally, the controls of the Interferon-gamma ELISA (Control 1 and 2) must be included on every 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 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	ACE-2 20x
12. Sample buffer ready for use	blue	1 x 100 ml	ACE-2 DILUENT
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. Instruction for use	-	1 booklet	-

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

- Corresponding stimulation tube set, e.g. SARS-CoV-2 IGRA stimulation tube set (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): recommended to warm the wash buffer
- Stop watch
- Centrifuge
- 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 following the first opening

After opening, the reagents are stable until the indicated expiry date when stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

Warnings and precautions

- For research use only. Not for use in diagnostic procedures.
- The product must only be used by trained laboratory personnel in a clinical or research laboratory.
- If the packed reagents are visibly damaged, do not use the test kit.
- Before using the product, read the instruction for use carefully. Only the valid version is to be used.
- Do not substitute or mix the EUROIMMUN reagents with 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 controls of human origin have tested negative for HBsAg, anti-HCV and anti-HIV. Nonetheless, all reagents should be treated as being a potential infection hazard and should be handled with care.

Preparation and stability of the samples

- **Sample material:** Human lithium-heparin plasma (obtained after stimulation e.g. using the SARS-CoV-2 IGRA stimulation tube set (EUROIMMUN order no. ET 2606-3003)
- **Notes on sample handling:** Plasma should be stored in polypropylene tubes. When lithium-heparin 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 -20 °C. Plasma samples are stable for 3 months at -20 °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:**

  If the ELISA measurement will not be performed immediately after stimulation: Preparation of stored stimulated plasma samples

  Bring the stimulated plasma samples to be investigated to room temperature (+18 °C to +25 °C) before the analysis and centrifuge for 10 minutes at 12.000 x g (Due to the storage, fibrin clots may have formed which can block the pipette tip if the samples are not centrifuged).

Manual processing of stimulated plasma samples:

Following centrifugation, dilute the plasma samples 1:5 in sample buffer; pipette the sample buffer into the tube first.

Example: Add 25 µl of sample to 100 µl sample buffer and mix well by vortexing (Sample pipettes are not suitable for mixing.).

Automated processing of stimulated plasma samples:

Following centrifugation, load the plasma samples into the EUROIMMUN analysis instruments.

If the ELISA measurement is performed immediately after stimulation: Preparation of whole-blood samples

Centrifuge the stimulated whole-blood samples in the EUROIMMUN stimulation tubes which are to be investigated at 12,000 x g for 10 minutes before the analysis.

Manual processing of stimulated whole blood:

Following centrifugation, dilute the plasma obtained from the stimulated whole-blood samples 1:5 in sample buffer; pipette the sample buffer into the tube first.

Example: Add 25 µl of sample to 100 µl sample buffer and mix well by vortexing (Sample pipettes are not suitable for mixing.).

Automated processing of stimulated whole blood:

Following centrifugation, load the stimulated whole-blood samples in the stimulation tubes into the EUROIMMUN analysis instruments.

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) approx. 30 minutes 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:** Lot-specific. Reconstitute calibrators with 1 ml deionised or distilled water approximately 10 minutes before use, invert them and mix thoroughly upside down. 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 -20 °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 are stable for up to three months at -20 °C. They can be frozen and thawed up to six times.
- **Biotin:** Lot-specific. Ready for use. Mix the biotin thoroughly before use.
- **Enzyme conjugate:** Lot-specific. Ready for use. Mix the reagent thoroughly before use.
- **Sample buffer:** 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 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

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 1 and 2 must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls 1 and 2 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), 87/586, Non WHO Reference Material). The quantification is made in international units per mililiter (mIU/ml).

Assay procedure

(Partly) manual test performance

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

Sample incubation: (1st step)
Transfer **100 µl** of the **calibrators, controls and diluted 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. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Conjugate incubation: (3nd 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. 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.

Test performance using fully automated analysis devices

Sample dilution and subsequent test processing can be fully automated by means of EUROIMMUN analysis instruments (EUROIMMUN Analyzer I, EUROIMMUN Analyzer I-2P, EUROLabWorkstation (ELW) ELISA).

The automated processing is intended for research use only and does not constitute a diagnostic application.

The incubation conditions given in the respective software authorised by EUROIMMUN may deviate slightly from those given in the test instruction of the ELISA.

Note: Samples must be centrifuged prior to loading (10 minutes at 12,000 x g) and must not be mixed afterwards! We recommend loading the analysis instruments according to a defined scheme.

Pipetting protocol

For manual and automated test performance on the EUROIMMUN analysis instruments, the following pipetting scheme is recommended:

	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	--

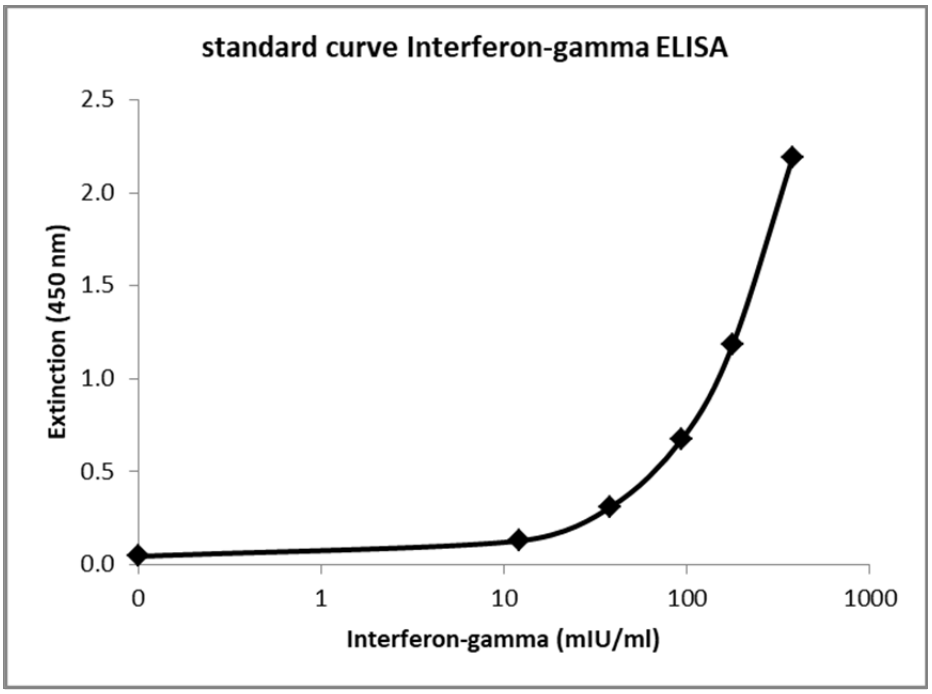
The pipetting scheme above applies to the quantitative analysis of plasma samples obtained using a EUROIMMUN stimulation tube set (e.g. EUROIMMUN SARS-CoV-2 IGRA stimulation tube set order no. ET 2606-3003). The numbering from 1 to 29 refers to the whole-blood samples used for the test.
It is **mandatory** to incubate the plasma samples of a stimulation tube set of one whole blood sample on one plate so that the subtraction of the Interferon-gamma BLANK can be correctly performed for the conditions STIM and TUBE.

The controls 1 and 2 (Co 1 and Co 2) serve as internal controls for the reliability of the Interferon-gamma ELISA measurement. They must be assayed with each test run.

Test evaluation

Quantitative: The standard curve from which the concentration of antibodies in the patient 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. When the EUROIMMUN programmings for Magellan/Analyzer/ELW are applied, this step is not required.

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 the calibrator 6, we recommend preparing a 1:10 predilution with sample buffer for the sample and remeasuring it in a new test run according to the incubation instructions. When preparing the dilution, first pipette the sample buffer into the polypropylene tubes (e.g. 108 µl) and then add the sample (e.g. 12 µl). This dilution must be taken into account in the subsequent calculation.

With duplicate determinations, the mean value should be used for the calculations. If the values from a duplicate determination deviate substantially from one another, EUROIMMUN recommends retesting the sample.

Note: Please note that such remeasurement requires for the three samples of a stimulation tube set to be diluted correspondingly and be remeasured together.

Specific information for the evaluation of results for plasma obtained using the EUROIMMUN stimulation tube set, e.g. SARS-CoV-2 IGRA stimulation tube set (ET 2606-3003):

The concentration of interferon-gamma in the plasma of the BLANK tube represents the individual interferon-gamma background and must therefore be manually subtracted from the interferon-gamma concentration of the plasma obtained from the conditions STIM and TUBE. This BLANK subtraction must be made individually for the conditions STIM and TUBE of every whole-blood sample. If the interferon-gamma concentrations were not determined using a EUROIMMUN software, the sample dilution must first be taken into account before subtracting the BLANK concentration.

After the BLANK subtraction, the concentration of interferon-gamma concentration in the STIM condition must be sufficiently higher than the BLANK value itself in order for a sufficient number and stimulability of immune cells in the whole-blood sample to be considered as given.

Note: The interferon-gamma concentrations of plasma obtained from STIM or TUBE of the EUROIMMUN SARS-CoV-2 IGRA stimulation tube set (order no. ET 2606-3003) may lie above the concentration of calibrator 6 or outside of the measurement range of the microplate. In these cases, the EUROIMMUN programmings issue the defined numeric value 2500 mIU/ml for the undiluted sample.

The concentration of plasma obtained from BLANK may as well lie below the concentration of calibrator 1. In these cases, the EUROIMMUN programmings issue the defined numeric value 0.5 mIU/ml for the undiluted sample.

The mentioned defined numeric values can also be applied as a basis for non-software-supported calculations by the user.

The test results are not affected in these cases and **no remeasuring** with another dilution must be performed.

Analytical performance

Measurement range:

Limit of blank (LoB): 1.22 mIU/ml
Limit of detection (LoD): 3.88 mIU/ml

Reproducibility: Studies on the reproducibility were carried out according to 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	1138.20 mIU/ml		630.36 mIU/ml		346.23 mIU/ml		257.06 mIU/ml		124.75 mIU/ml		83.39 mIU/ml	
	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Repeatability	32.199	2.8%	16.323	2.6%	13.257	3.8%	9.807	3.8%	3.781	3.0%	3.636	4.4%
Between run	40.849	3.6%	17.176	2.7%	6.290	1.8%	5.553	2.2%	3.482	2.8%	2.469	3.0%
Between day	20.507	1.8%	4.119	0.7%	2.269	0.7%	2.685	1.0%	3.318	2.7%	2.957	3.5%
Within lot	55.910	4.9%	24.050	3.8%	14.848	4.3%	11.585	4.5%	6.118	4.9%	5.297	6.4%
Between lot	20.397	1.8%	0.000	0.0%	8.827	2.5%	4.464	1.7%	4.571	3.7%	4.927	5.9%
Reproducibility	59.514	5.2%	24.050	3.8%	17.274	5.0%	12.416	4.8%	7.637	6.1%	7.235	8.7%

Precision: Studies on the intra-lab precision were carried out according to 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	
	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%

Linearity: The linearity of the Interferon-gamma ELISA was investigated according to CLSI guideline EP06-A. The Interferon-gamma ELISA is linear at least in the tested concentration range (1.24 mIU/ml to 1340.37 mIU/ml).

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to concentrations of 10 mg/ml haemoglobin, 20 mg/ml triglycerides and 0.4 mg/ml bilirubin in this ELISA.

Limitations of the procedure

- For research use only. Not for use in diagnostic procedures.
- The pipetting volumes, incubation times, temperatures, and preparation steps given in the instruction for use must be adhered to.
- Correct performance of sample collection and storage is crucial for the test results.
- The interferon-gamma concentration can only be correctly determined if all plasma samples 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 using a thermostatically adjusted ELISA incubator in all incubation steps. The higher the room temperature during the incubation steps, the greater will be the extinction. The same variations also apply to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
- 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 extinction readings.

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).

Meaning of the symbols

Symbol	Meaning	Symbol	Meaning
STRIPS	Microplate strips	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	RUO	For research use only
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