Instructions for use for the
SARS-CoV-2 IGRA stimulation tube set

For research use only RUO

ORDER NO.	ANTIBODIES AGAINST	SUBSTRATE	FORMAT
ET 2606-3003	SARS coronavirus 2 (SARS-CoV-2)	Ag-coated stimulation tubes	30 x 01 (30)

Antigen

The CoV-2 IGRA TUBE used in this stimulation tube set is coated with antigens based on the S1 domain of the SARS-CoV-2 spike protein.

Test principle

These stimulation tube sets must only be used together with the EUROIMMUN Interferon-gamma ELISA (EUROIMMUN order no. EQ 6841-9601).

The package contains stimulation tube sets consisting of three stimulation tubes for use with one sample. The stimulation tubes are used for the treatment of whole blood to obtain plasma. Subsequently, the concentration of the released interferon-gamma is measured in the plasma.

1. CoV-2 IGRA BLANK, transparent cap: The stimulation tube contains no activating components for immune cells. Thus, no interferon-gamma secretion is induced. The plasma thus obtained is used for the determination of the individual interferon-gamma background.
2. CoV-2 IGRA TUBE, yellow cap: The stimulation tube is coated with components of the S1 domain of the SARS-CoV-2 spike protein.
3. Cov-2 IGRA STIM, violet cap: The stimulation tube is coated with a mitogen causing an unspecific interferon-gamma secretion. The plasma thus obtained is used to verify whether the sample contains immune cells in a sufficient quantity and with a sufficient ability to be activated.

Fresh human whole blood from lithium-heparin blood collection tubes is pipetted into the three stimulation tubes and incubated. If immune cells capable of being activated are present, they are stimulated to secrete interferon-gamma during the incubation. After incubation of the whole blood in the stimulation tubes, centrifugation is performed to collect heparin plasma, which is then used for the determination of the interferon-gamma concentration. The procedure is described in the test instruction of the corresponding test kit (EQ 6841-9601).

The interferon gamma concentration in the plasma of the BLANK represents the individual interferon-gamma background and is therefore to be subtracted from the interferon-gamma concentration of the plasma obtained from the conditions TUBE and STIM. This subtraction of the BLANK must be performed individually for the conditions TUBE and STIM of each whole-blood sample.

After BLANK subtraction, the interferon-gamma concentration in the STIM condition must still be sufficiently higher than the BLANK itself to consider a sufficient quantity and stimulation ability of the immune cells in the whole-blood sample as given.

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

- Calibrated pipettes
- Pipette tips
- Incubator for incubation of the stimulation tubes in an upright position at +37 °C
- Centrifuge with insert for 2-ml polypropylene screw cap microtubes
- Polypropylene screw cap microtubes with screw caps for storage of the stimulated plasma if the interferon gamma concentration is not measured directly after the stimulation
- EUROIMMUN Interferon-gamma ELISA (order number EQ 6841-9601)

Storage and stability

The package must be stored at +2 °C to +8 °C; do not freeze! Unopened, the stimulation tubes are stable until the indicated expiry date.

Warnings and precautions

- The product must only be used by trained laboratory personnel in a clinical or research laboratory.
- Before starting the procedure, read the instructions carefully. Only the valid version is to be used.
- Do not substitute or mix the EUROIMMUN reagents with reagents from other manufacturers.
- Observe Good Laboratory Practice (GLP) and safety guidelines. 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.

Preparation and stability of the samples

- **Samples:** heparinised whole blood (lithium heparin)
- **Sample preparation:** Carefully tilt the blood collection tubes containing the whole blood several times before transferring the whole blood into the stimulation tubes.
- **Note:** Only use stimulation tubes filled with 500 µl of fresh whole blood for the analysis. Do not vortex the blood collection and stimulation tubes.
- **Stability of the samples:** Use heparinised whole blood up to maximally 16 hours after venous puncture. The whole blood should generally be used as soon as possible after collection. Store the whole blood at room temperature (+18 °C to +25 °C) until use; do not cool!

Preparation and stability of the reagents

Note: The stimulation tubes must be brought to room temperature (+18°C to +25°C) before use**.**

The thermostatically adjustable ELISA incubator must be set to +37°C ± 1°C.

Waste disposal

Human material should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Procedure of the stimulation of immune cells in heparinised whole blood for interferon-gamma secretion:

1. Warm one SARS-CoV-2 IGRA stimulation tube set (1x CoV-2 IGRA BLANK, 1x CoV-2 IGRA TUBE, 1x CoV-2 IGRA STIM) per whole-blood sample to room temperature (+18 °C to +25 °C)

   Note: The stimulation tubes may either appear empty or contain droplets.

2. Carefully tilt the lithium-heparin blood collection tube with whole blood from side to side to mix the whole blood evenly.

3. Remove the caps from the stimulation tubes and add 500 µl of whole blood to each tube.

   Note: After each stimulation tube, change the pipette tip to avoid antigen carry-over between the different stimulation tubes of one set!

   Recommendation: Pipette the whole blood in the following order: 1. CoV-2 IGRA BLANK, 2. CoV-2 IGRA TUBE, 3. CoV-2 IGRA STIM.

4. **Tightly seal** the stimulation tubes with the corresponding caps.

5. **Rapidly invert** the filled stimulation tubes **6x** to fully detach any coating on the walls or bottom of the tube. Do not vortex!

6. The entire surface of the stimulation tube must be covered with whole blood. Otherwise, invert the filled stimulation tubes again.

7. After mixing, seal the stimulation tubes tightly and incubate at +37 °C ± 1 °C in an upright position for 20 to 24 h.

8. Withdraw of the filled stimulation tubes from the incubator.

9. Stop the stimulation and determine the interferon-gamma concentration in the samples using the EUROIMMUN Interferon-gamma ELISA (order no. EQ 6841-9601)

a) For subsequent ELISA measurement refer to the section “Sample preparation” in the test instruction of the Interferon-gamma ELISA (order no. EQ 6841-9601).

b) Storing of the plasma if the ELISA measurement is not performed immediately after the stimulation:

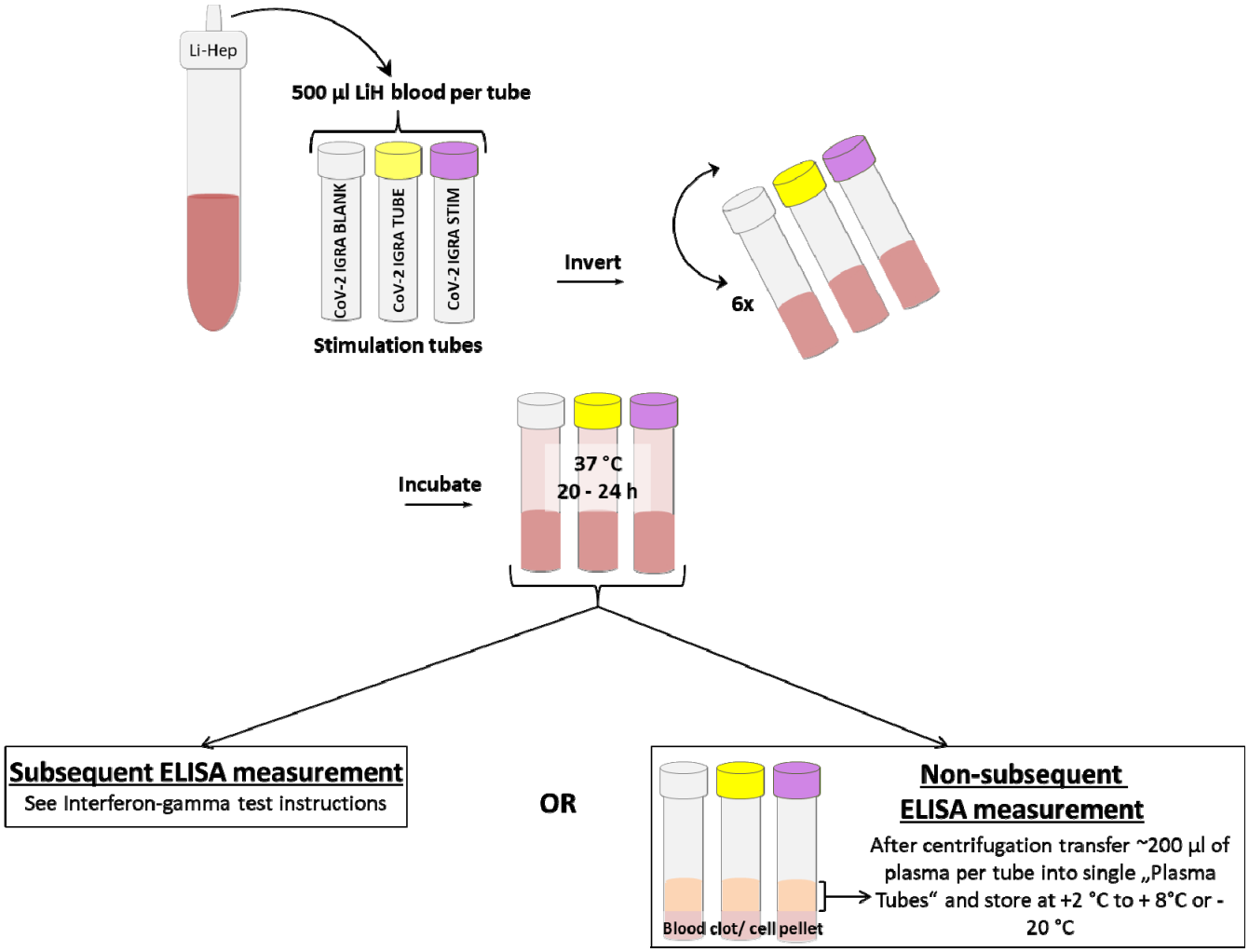
   Centrifuge the filled stimulation tubes for 10 min at 12,000 x g.

   For each stimulation tube, carefully separate the plasma (approx. 200 µl) from the top and transfer into a new polypropylene screw-cap microtube (“plasma tube”), avoiding contamination of the plasma with blood clot components.

   The plasma is stable for up to 28 days at + 2 °C to + 8°C and for up to three months at -20 °C.

   For non-subsequent ELISA measurement refer to the section “Sample preparation” of the test instruction of the Interferon-gamma ELISA (EUROIMMUN file no. EQ_6841_A_UK_BXX).

Schematic representation



Pipetting scheme

It is recommended that the plasma samples from the EUROIMMUN SARS-CoV-2 IGRA stimulation tube set for measurement with the EUROIMMUN Interferon-gamma ELISA (order no. EQ 6841-9601) be pipetted according to a defined scheme to avoid a mix-up of the three plasma samples belonging to one whole-blood sample. The plasma samples of one set 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.

Further information on the interferon-gamma measurement and evaluation can be found in the test instruction of the EUROIMMUN Interferon-gamma ELISA (order no. EQ 6841-9601).

Limitations of the procedure

- The EUROIMMUN SARS-CoV-2 IGRA stimulation tube set is exclusively tested for use with human lithium-heparin whole blood.
- The whole blood must be stored at room temperature until stimulation (including transport) and stimulated at the latest 16 hours after venous puncture using the EUROIMMUN SARS-CoV-2 IGRA stimulation tube set.
- The activation of the immune cells to secrete interferon gamma depends directly on the incubation temperature and stimulation time, which must therefore be strictly observed.
- Stimulation tubes must not be frozen.

Technical support

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

Further information can be found in the test kit instruction of the EUROIMMUN Interferon-gamma ELISA (order number EQ 6841-9601).

Meaning of the symbols

Symbol	Meaning	Symbol	Meaning
CoV-2 IGRA BLANK	Stimulation tube without stimulating coating		Unopened usable until (YYYY-MM-DD)
CoV-2 IGRA STIM	Stimulation tube coated for unspecific stimulation of an interferon-gamma secretion		Manufacturing date (YYYY-MM-DD)
CoV-2 IGRA TUBE	Stimulation tube coated with antigens of the SARS-CoV-2 spike protein		Manufacturer
	Lot description		Observe instructions for use
	For research use only		Order number
	Storage temperature		Contents suffice for <n> analyses