immunoserv

# Immuno-T™ Laboratory Test Kit

INSTRUCTIONS FOR USE

## Principle of the Test

The Immuno-T™ test is used to determine whether an individual possesses T cell reactivity to the selected test antigens. This T cell response may have arisen through exposure, prior infection, vaccination, or antigen cross-reactivity. For example, prior scientific evidence indicates that the T cell response measured by the Immuno-T™ test can identify an individual's risk of testing positive for COVID-19 in the 6 months following the test.

T cells can be reactivated *in vitro* by exposure to antigens causing the release of Interferon-gamma (IFN-γ) into the blood plasma, which can be measured in a sample of plasma via Enzyme Linked Immunosorbent Assay (ELISA). Reactive T cells can be identified in fresh venous blood collected into lithium- or sodium-heparin blood collection tubes.

## Part I: Blood Collection and Stimulation

### Kit Contents:

| Stimulation Tubes | 20 test (1 plate kit) | 100 test (5 plate kit) |
|---|---|---|
| **Negative Control**<br>2 mL microcentrifuge stimulation tubes | 20 | 100 |
| **Positive Control**<br>2 mL microcentrifuge stimulation tubes containing lyophilised phytohemagglutinin (PHA) | 20 | 100 |
| **Test Antigen**<br>2 mL microcentrifuge stimulation tubes containing lyophilised test antigen(s) | 20 | 100 |

### Materials and equipment required and not supplied:

**a)** Precision calibrated micropipettes and filter tips capable of pipetting 100 µL - 1000 µL.

**b)** Microcentrifuge with insert for 2 mL microcentrifuge tubes.

**c)** 96 well conical or round-bottom plate (300 µL) for sample storage.

**d)** Incubator (37 °C).

**e)** Class II Biosafety Cabinet.

**f)** 2 mL microcentrifuge tube racks.

**g)** Fridge (2 - 8 °C) for storage of kits and plasma samples (plasma samples can be stored for up to 1 month post-stimulation).

**h)** Freezer (-20 °C), for long-term storage of plasma samples (up to 1 year post-stimulation).

## Protocol

**1.** Collect a minimum of 4 mL whole blood into a sodium- or lithium-heparin blood collection tube. Unstimulated blood should be stored at room temperature and sample(s) should be processed within 24 hours of blood draw.

**2.** Equilibrate a negative control, positive control & test antigen stimulation tube per donor to room temperature for a minimum of 15 minutes.

**3.** Label stimulation tubes with allocated donor ID and briefly centrifuge to bring contents to the bottom of the tube.

**Perform the following steps in a Class II Biosafety Cabinet:**

**4.** Gently invert blood collection tube to ensure cells are fully suspended directly prior to stimulation.

**5.** Transfer 1000 µL of whole blood into the relevant stimulation tube and gently pipette up and down five times to mix the blood. Repeat for each stimulation.

**6.** Incubate the stimulation tubes at 37 °C for 16-26 hours.

**7.** Following incubation, centrifuge stimulation tubes at 800 x g for 2 minutes at room temperature.

**8.** Transfer 150 µL of plasma from the stimulation tube, taking care not to disturb the pellet, into the assigned well of a round-bottom 96-well plate (A). Plasma samples can be stored at 2-8 °C for up to 1 month prior to performing the assay. A further 150 µL can be added to a second (B) plate for long term storage at -20 °C (up to 12 months). Ensure plates are sealed before storage.

## Part II: IFN-ɣ ELISA

### Kit Contents:

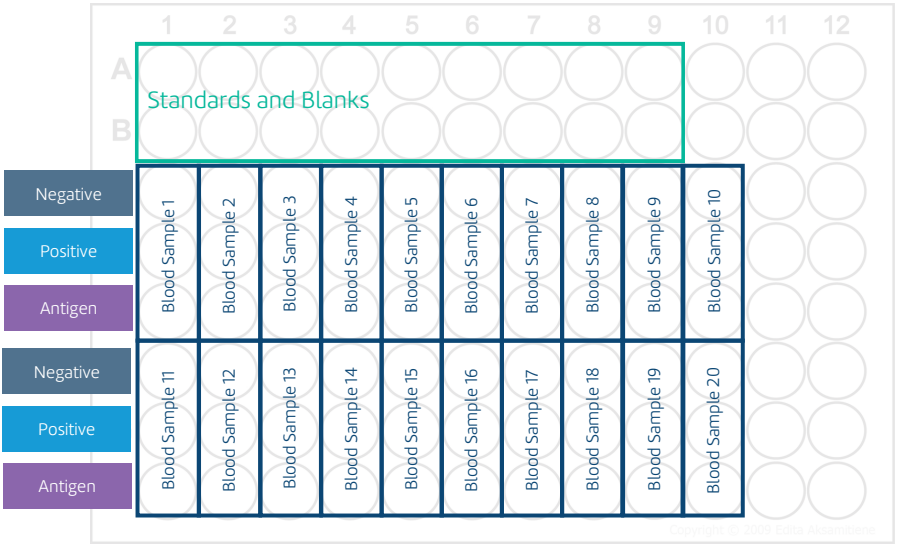
| Name | 20 test (1 plate kit) | 100 test (5 plate kit) |
|---|---|---|
| Human IFN-ɣ Capture Antibody (200X) | 60 µL | 300 µL |
| Human IFN-ɣ Detection Antibody (200X) | 60 µL | 300 µL |
| Human IFN-ɣ Standard | 1 vial | 1 vial |
| Avidin-HRP (1000X) | 14 µL | 60 µL |
| TMB Substrate Solution | 12 mL | 60 mL |
| Assay Diluent (5X) | 11 mL | 60 mL |
| Stop Solution | 30 mL | 30 mL (x2) |
| Wash Buffer (20X) | 25 mL | 110 mL |
| Coating Buffer (5X) | 2.5 mL | 30 mL |
| PBS (10X) | 5 mL | 25 mL |
| Plate Sealers | 7 | 35 |
| ELISA Assay Plate | 1 | 5 |

### Materials and equipment required but not supplied:

**a)** Precision calibrated micropipettes and tips capable of pipetting 1 µL – 1000 µL.
**b)** Serological pipettes and pipette controller.
**c)** Measuring cylinder.
**d)** Precision calibrated multi-channel pipette capable of pipetting 50 µL - 200 µL.
**e)** Reagent reservoirs.
**f)** 96-well conical or round-bottom plate transfer plate.
**g)** Deionised water.
**h)** Container (500 mL).
**i)** Absorbent paper towels.
**j)** Plastic container.
**k)** 15 mL and 50 mL tubes.
**l)** Microcentrifuge tubes.
**m)** Plate shaker capable of 500 rpm.
**n)** A microplate reader capable of reading at 450 and 620 nm (Recommended: BioLegend's Mini ELISA Plate Reader™ cat. no. 423555).
**o)** Fridge (2 - 8 °C).

**The following describes the procedure for analysis of 20 test samples on 1 plate.**

## Suggested Assay Plate Layout:




## Reagent Preparation:

Allow all reagents to equilibrate to room temperature for a minimum of 15 minutes and briefly centrifuge tubes prior to preparation. Ensure each solution is mixed thoroughly before use. All reagents should be diluted immediately prior to use.

| | |
|---|---|
| **PBS** | Dilute 5 mL of 10X PBS in 45 mL of deionised water for a final volume of 50 mL 1X PBS. |
| **Coating Buffer** | Dilute 2.2 mL of 5X Coating buffer in 8.8 mL of deionised water for a final volume of 11 mL 1X Coating Buffer. |
| **IFN-ɣ Capture Antibody** | Add 55 µL of 200X IFN-ɣ Detection Antibody to 11 mL 1X Coating Buffer to prepare a 1X working solution. |
| **Wash Buffer** | Dilute 20 mL of 20X Wash Buffer in 380 mL of deionised water for a final volume of 400 mL 1X Wash Buffer. |
| **Assay Diluent** | Dilute 10 mL of 5X Assay Diluent in 40 mL of 1X PBS for a final volume of 50 mL 1X Assay Diluent. |
| **IFN-ɣ Detection Antibody** | Add 55 µL of IFN-ɣ Detection Antibody to 11 mL 1X Assay Diluent per plate. |

**Avidin-HRP** Add 11 µL of 1000X Avidin-HRP to 11 mL 1X Assay Diluent per plate.

### IFN-ɣ Standard

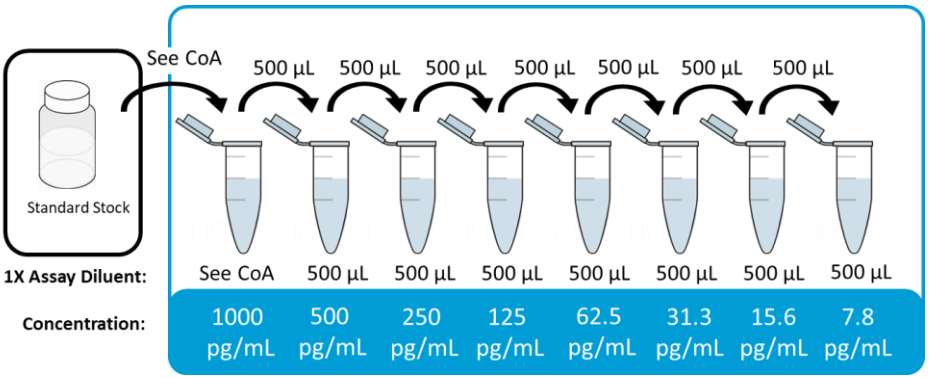
— Reconstitute the IFN-ɣ standard by adding 200 µL of 1X Assay Diluent to the vial to prepare a stock solution. Allow the standard to dissolve at room temperature for 15 minutes and mix thoroughly.

**NOTE: For the 100 test kit format, the reconstituted standard should be immediately aliquoted and stored at –20 °C for use in subsequent assays. Avoid freeze-thaw cycles.**

— Prepare standard curve samples within 30 minutes of use, taking care to mix thoroughly before any subsequent dilution and use a new primed tip for each dilution step.

— **Refer to lot-specific Certificate of Analysis for instructions on how to prepare the 1000 pg/mL top standard.**

— Prepare the seven microcentrifuge tubes by adding 500 µL of 1X assay diluent and perform seven two-fold serial dilutions using the diagram below for a total of eight standards. (1X Assay Diluent will serve as a blank)




**Standard Curve**

## Assay Protocol

### Day 1

1. Add 100 µL per well of IFN-ɣ Capture Antibody solution to the assay plate.
2. Seal the plate and incubate at 2 - 8 °C overnight.

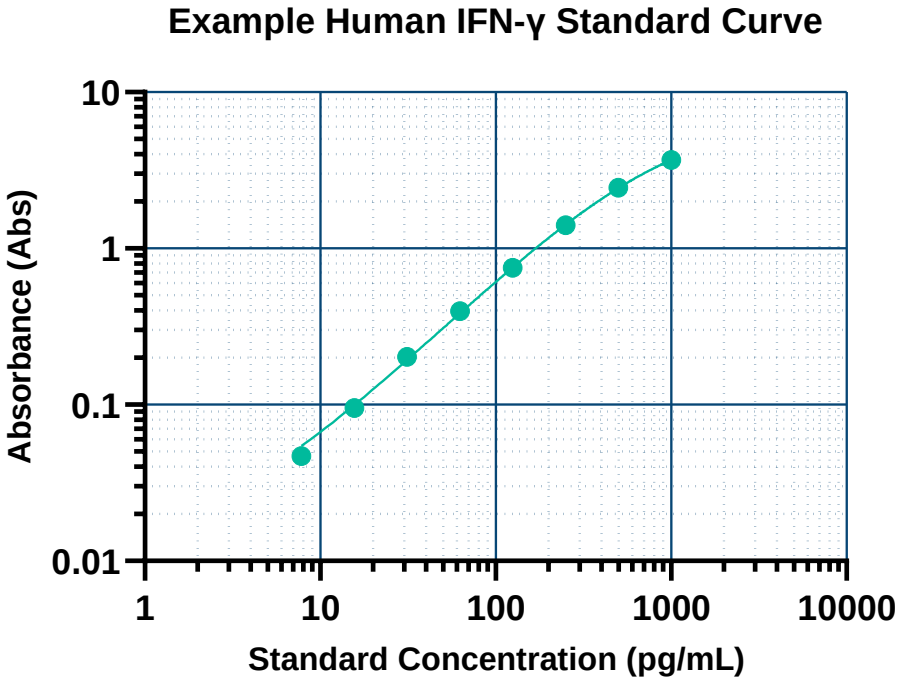
### Day 2

1. Empty the contents of the assay plate into a container lined with absorbent paper towels. Wash the plate by adding 150 µL of Wash Buffer to each well and tipping Wash Buffer out of plate into a container lined with a fresh layer of absorbent paper to prevent splashing. On the bench, blot onto a separate piece of absorbent paper to remove excess Wash Buffer. Repeat this procedure 3 times for a total of 4 washes.
2. Block non-specific binding to the assay plate by adding 200 µL of 1X Assay Diluent to each well, seal the plate with a fresh plate seal and incubate at room temperature for 60 minutes with shaking (500 rpm).
3. During the blocking step prepare the standards and equilibrate plasma samples to be analysed to room temperature.
4. Transfer a minimum of 120 µL per well of each of the plasma samples, standards and 1X Assay Diluent blank to a transfer plate according to the plate map (standards and assay blank should be run in duplicate) and place to one side.
5. Following incubation, wash the assay plate 4 times according to the procedure outlined in step 1.
6. Transfer 100 µL of each sample from the transfer plate, to the corresponding wells of the assay plate. Seal the assay plate with a fresh plate seal and incubate at room temperature for 60 minutes with shaking (500 rpm). Dispose of the transfer plate.
7. Wash the assay plate 4 times according to the procedure outlined in step 1.
8. Add 100 µL per well of Detection Antibody Solution to the assay plate, seal the plate with a fresh plate seal and incubate at room temperature for 60 minutes with shaking (500 rpm).
9. Wash the plate 4 times according to the procedure outlined in step 1.
10. Add 100 µL per well of Avidin-HRP, seal the plate with a fresh plate seal and incubate at room temperature for 30 minutes with shaking (500 rpm).
11. Wash the plate 5 times according to the procedure outlined in step 1.
12. Add 100 µL of TMB substrate solution to each well, seal with a fresh plate seal and incubate at room temperature in the dark for 15 minutes (place onto a flat surface in a drawer or cupboard to protect from light).
13. Add 100 µL of Stop Solution to each well and measure Absorbance at 450 nm with a reference wavelength of 620 nm using a plate reader. The plate must be read within 15 minutes of adding the Stop Solution.

## Calculation of Results

Results are best calculated with computer software (BioLegend Mini ELISA reader software is recommended if using the BioLegend Mini ELISA Plate Reader™) using a 5 or 4 parameter logistics curve-fitting algorithm. If plasma samples have been diluted, multiply result by the dilution factor. If a test falls outside the standard curve range, the value can either be reported as <7.8 or >1000 pg/mL or if above the standard curve, the remaining plasma sample can be diluted and re-analysed.

## Typical Data



# Troubleshooting

**Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.**

### High background:

- Contaminated blank wells.
- Matrix used contained endogenous analyte.
- Insufficient plate washing.
- Contamination of TMB substrate solution.

### No signal:

- Incorrect concentration of antibody added to the plate.
- Avidin HRP not added.
- TMB Substrate solution was not added.
- Wash Buffer contained sodium azide.

### Low or poor signal for the standard curve:

- Standard incompletely reconstituted or stored improperly.
- Reagents were added to wells at incorrect concentrations.
- Plate was incubated with improper temperature, timing or agitation.

### Signal too high, standard curve saturated:

- Standard reconstituted with lower volume than instructed.
- One or more reagent steps incubated for too long.
- Plate incubated with inappropriate temperature, timing, or agitation.

### Sample readings out of range:

- Samples contain no analyte or levels below limit of detection.
- Samples contain concentration of analyte greater than upper limit of detection (above top standard).

### High sample/standard variation:

- Pipetting errors.
- Inadequate or non-uniform plate washing.
- Samples not homogenised before transfer to the plate.
- Contamination of sample or standard wells.

## Technical Support

In case of questions or technical issues, contact info@immunoserv.com or call +44 2922 806 314 Monday-Friday between 9am and 5pm (UK).

## Safety Information

Blood samples should be handled according to local guidelines.
Reagents may contain preservatives that can be harmful if ingested, inhaled, or absorbed through the skin. Handle all reagents with care and maintain laboratory hygiene. For more information see MSDS.

### Hazard Statement

May cause allergic reaction to the skin.

Stop solution bottle contains strong acid. May cause severe skin burns and eye damage.

### Precautionary Statements

Wear protective gloves / eye protection.

Wear protective clothing.

Avoid breathing mist / vapours / spray.

If skin irritation or rash occurs, seek medical attention immediately.

IF ON THE SKIN: Wash with plenty of water.

Dispose of contents / containers in accordance with local regulations.

## NOTES

Please email **Info@ImmunoServ.com** for any additional support.

**immunoserv.com**

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