

## Simian M. tuberculosis Interferon-Gamma release assay (IGRA)

### Test Kit Instructions

5083001-2023(03)

The Simian M. tuberculosis (TB) Interferon - Gamma release assay (IGRA) Test Kit (Cat#5083001) is a qualitative test designed to detect TB as a supplement or alternative to the Tuberculin Skin Test (TST).

This kit is divided into two parts: ①Addition of

Stimulation Antigens and incubation; ②TB Blood Test. Measure the T cell-mediated immune response (the release of interferon gamma, IFN- $\gamma$ ) from mixing *Mycobacterium tuberculosis* antigens with whole blood in vitro.

#### Materials (48 tests)

	Contents	Quantity	Description
1	Medium	5 ml×4	2-8°C, ready-to-use
2	Negative stimulant N (N)	1.5 ml×2	2-8°C, ready-to-use
3	Positive stimulant P(P) *	150 $\mu$ l×2	-20°C, working solution: A(v):medium(v)=1:8
4	Stimulant A(A)	300 $\mu$ l	2-8°C, working solution: A(v):medium(v)=1:8
5	Stimulant B(B)	300 $\mu$ l	2-8°C, working solution: B(v):medium(v)=1:8
6	Coated Plate	2 plate	2-8°C
7	Concentrated Wash Buffer (20x)	50 ml	RT, working solution: add dH <sub>2</sub> O to dilute 20 times
8	10xSample Dilutant	4 ml	2-8°C
9	1xPBS Buffer	50 ml	RT, ready-to-use
10	Biotinylated Ab	24 ml	2-8°C, ,ready-to-use
11	Streptavidin-HRP	24 ml	2-8°C, ,ready-to-use
12	Positive Control	1 ml	2-8°C, ready-to-use
13	Negative Control	1 ml	2-8°C, ready-to-use
14	SureBlue Substrate	24 ml	2-8°C, ready-to-use
15	Stop Solution	24 ml	RT, ready-to-use
16	48-well cell culture plate.	/	RT

NOTE: \* Reagents 3 Positive stimulant P(P) are packed and shipped separately from other reagents. Avoid repeated freezing and thawing.

#### Additional Materials Required

1. Distilled water (dH<sub>2</sub>O) or deionized water.
2. Centrifuge tubes (2ml, 15ml and 50ml) and Reagent bottles.
3. Deep-well plates or tubes.
4. Vortex mixer and Timer.
5. Validated Microplate Strip Washer and Centrifuge
6. Absorbance Microplate Reader (450 nm & 620-650 nm).
7. Validated adjustable micropipettes, single (10, 100 and 1000  $\mu$ l) and multichannel pipette (50-300  $\mu$ l) and tips.
8. Lithium heparin vacutainers.
9. Carbon dioxide incubator.

#### Blood Collection and Preservation

1. Collect a minimum volume of 2 ml blood from each animal into a blood collection tube containing heparin as anticoagulant. Gently mix the blood by inverting the tubes 5~10 times to dissolve the heparin.
2. Blood samples should be transported to laboratory at ambient temperature (18-25°C), its maximum temperature shall not exceed 36°C, and culture within 24 hours of collection. It is not recommended to store the blood in the refrigerator.
3. Use a new pipette tip for each plasma sample.

Plasma can be stored at 2-8°C for up to 7 days if not required for assays on the day of collection. For longer periods, samples may be frozen at -20°C for several months. Avoid repeated freeze or thaw cycles.

## Reagent preparation:

1. Equilibrate all the reagents to room temperature for at least 30 min before use.
2. **Prepare Wash Buffer (0.05%PBST, pH 7.2~7.4):**  
Check the wash concentrate for the presence of salt crystals. If crystals are observed in the solution, warm at 37°C until crystals have completely dissolved. Wash Buffer is stable for 2 weeks from the date of preparation (store at room temperature). Dilute the Concentrated Wash Buffer 1:19 with deionized or distilled water in a clean glass or plastic screw cap container to make 0.05% PBST wash buffer. Mix gently by inverting the container several times (avoid excessive foaming).
3. **Prepare Sample Dilutant:**  
Dilute the 10×Sample Dilutant 1:9 with 1XPBS Buffer in a clean Centrifuge tubes to make 1×Sample Dilutant. Each animal requires about 200 µl sample dilutant solution.
4. **Prepare Stimulant A/B/P(A/B/P):**  
Dilute the **Stimulant A/B/P(A/B/P)** Calculate the required volume of **A/B/P** working solution based on the number of samples, 50 µl per animal for each type working solution.1:8 with Medium in a clean Centrifuge tubes to make working solution.

## Test Procedure

1. **Addition of Stimulation Antigens:**  
**For N/A/B/P:** Add **50 µl** of N, diluted working stock of A, B and P aseptically to the appropriate wells of a 48-well tissue culture tray.  
**Dispense blood:** Blood samples must be evenly mixed before making aliquots. Use a roller-rocker or gently invert tubes about 10 times immediately prior to dispensing the aliquots. Dispense four **500 µl** aliquots of heparinized blood from each animal into the appropriate wells containing the stimulation antigens previously dispensed as per the procedure mentioned above. This should be performed under aseptic conditions using either sterile disposable pipettes with automatic pipette filler or sterile transfer pipettes. The antigens must be mixed thoroughly into the aliquoted blood.  
**NOTE:** It is important to keep cell damage to an absolute minimum as the test requires viable lymphocytes.
2. **Incubation:**  
Incubate tissue culture trays, containing blood and antigens, for **16-24** hours at **37°C** in a carbon dioxide incubator with **5% CO<sub>2</sub>** (Fresh blood samples can be incubated using a incubator without CO<sub>2</sub>).

## 3. Plasma preparation:

10. Plasma collection may be facilitated by centrifuging the 48-well trays at **500 xg** for **10 minutes** at room temperature (18-25°C). After the centrifuge, carefully collect plasma (supernatant) and transfer into Deep-well plates or tubes.

**NOTE:** It is important to minimize collection of any cellular material along with plasma. However, contamination of the plasma with a very small amount of erythrocytes during harvesting has no effect on the IFN-γ ELISA. Similarly, slight hemolysis of blood samples has little effect on the IFN-γ ELISA.

## 4. Specimen Incubation

Set up one negative control well and one positive control well in each individual test run. Add 100 µl each of provided controls (Ready-to-use) into its assigned well. Pipet 50 µl of 1×Sample Dilutant into each microplate well. Add 50 µl of test samples into its assigned well, seal the plate with an adhesive seal and mix well gently. Cover with an adhesive strip and incubate for **2 hours** at room temperature.

5. Aspirate the liquid of each well and wash the plate **3 times with 350 µl** of Wash Buffer.
6. Add **100 µl** of Biotinylated Ab to its designated wells; incubate for **1 hour** at room temperature.
7. Aspirate the liquid of each well and wash the plate **3 times with 350 µl** of Wash Buffer.
8. Add **100 µl** of Streptavidin-HRP to each well; incubate for **1 hour** at room temperature and keep the plate away from direct light.
9. Aspirate the liquid if each well and wash the plate **3 times with 350 µl** of Wash Buffer.
10. Add **100 µl** of Sureblue substrate to each well; incubate for **30 minutes** and keep away from direct light.
11. Add **100 µl** of Stop Solution to each well immediately.
12. Read absorbance at **450nm/620-650nm** in 2 to 15 minutes.

## Results and Interpretation

The test result is valid when the negative control OD<0.2, and the positive control OD≥1.2.

Results	OD value		
	P-N	B-A	B-N
Positive	≥0.5	≥0.16	≥0.16
Indeterminate	≥0.5	0.10≤OD<0.16	
Negative	≥0.5	<0.10	<0.10
Pending	<0.5	/	/

NOTE: Each sample has four OD values: N, A, B and P. If the result is indeterminate or pending, please collect blood and test again. If A-N value of ≥ 0.16 may infect environmental mycobacteria.