Primagam™ Non-Human Primate M. tuberculosis Interferon-Gamma Kit

ELISA for detection of tuberculosis in non-human primates

Catalog Number 63311

Pub. No. MAN0018635 **Rev.** A.0

Technology	Species	Sample type
ELISA	Non-human primates	Whole blood

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Applied Biosystems™ Primagam™ Non-Human Primate M. tuberculosis Interferon-Gamma Kit is an ELISA-based diagnostic test that measures interferon-γ (IFN-γ) in whole blood samples to detect tuberculosis (TB). Release of IFN-γ in response to stimulation with tuberculin purified protein derivative (PPD) antigens is a highly predictive biomarker of TB infection. The kit detects IFN-γ in the following species: *Aotus* (night/owl monkey), *Ateles* (spider monkey), *Colobus* (guerezas), gibbon/siamang, guenon/De Brazza's, langur, lemur, macaque, mandrill, marmoset, squirrel monkey, tamarin, and vervet.

Tuberculosis is a progressive disease that affects the respiratory systems of primates. It can be transmitted from non-human primates to their caretakers in zoos and research laboratories, which presents a public health hazard. The Intradermal Eyelid Skin Test is the traditional method that is used to test for TB in non-human primates. This test requires immobilization and detects TB based on inflammation and swelling at the site of injection. The Primagam™ Non-Human Primate M. tuberculosis Interferon-Gamma Kit can be used for the detection of TB in addition to or as an alternative to the Intradermal Eyelid Skin Test.

The kit follows a two-day laboratory procedure:

- **Day 1**—Whole blood samples are collected in heparin tubes, then aliquots of each sample are incubated with a single antigen: Bovine PPD, Avian PPD, or Nil Antigen Control.
- **Day 2**—IFN-γ that is produced by antigen-stimulated lymphocytes is measured using ELISA technology.

The kit was evaluated during a tuberculosis outbreak in 54 cynomolgus (*Macaca fascicularis*) and 22 rhesus (*Macaca mulatta*) macaque monkeys. The results of the kit revealed 92% sensitivity with 100% specificity. An extra evaluation in a separate colony, presumed free of TB based on history, and a regular skin testing regime, also resulted in 100% specificity.

Contents and storage

Reagents and plates for 30 tests are supplied.

Note: For reconstitution and dilution instructions, see "Before you begin" on page 3.

Table 1 Primagam™ Non-Human Primate M. tuberculosis Interferon-Gamma Kit (Cat. No. 63311)

Contents	Description	Amount	Storage [1]
Microplate Strip[2]	Ready-to-use.	2 × 96-well microplates	2–8°C
Bovine PPD	Dilute before use.	5 mL	
Avian PPD	Dilute before use.	5 mL	
Nil Antigen Control	• Ready-to-use. • Contains 0.01% weight by volume (w/v) thimerosal.	5 mL	
Positive Primate IFN-γ Control	• Reconstitute before use. • Contains 0.01% w/v thimerosal.	1 vial of lyophilized solids	
Negative Primate IFN-γ Control	• Reconstitute before use. • Contains 0.01% w/v thimerosal.	1 vial of lyophilized solids	
Green Diluent (sample and Conjugate diluent buffer)	• Ready-to-use. • Contains 0.01% w/v thimerosal.	40 mL	
Conjugate-100x Concentrate (horseradish peroxidase-labeled anti-primate IFN-γ antibody)	• Reconstitute before use. • Contains 0.01% w/v thimerosal.	1 vial of lyophilized solids	
Wash Buffer-20x Concentrate	• Dilute before use. • Contains 0.01% w/v thimerosal.	2 × 50 mL	
Enzyme Substrate Buffer	• Ready-to-use. • Contains H_2O_2.	30 mL	
Chromogen Solution-100x Concentrate	• Dilute before use. • Contains TMB in DMSO.	0.5 mL	
Enzyme Stopping Solution	• Ready-to-use. • Contains 0.5M H_2SO_4.	15 mL	

[1] See label for expiration date.
[2] Wells are coated with antibodies directed against primate IFN-γ.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source[1]
Instruments	
Multiskan™ FC Microplate Photometer, or equivalent plate reader[2]	51119000
Wellwash™ Microplate Washer, or equivalent	5165000
(Optional) Microplate shaker	MLS
(Optional) Tube rocker	MLS

Item	Source[1]
Equipment	
Biosafety cabinet (BSC, level II)	MLS
Single channel pipette (10–1000 µL)	MLS
Multichannel pipette (50–500 µL)	MLS
Laboratory mixer (vortex or equivalent)	MLS
Needle holders, 2–3 per collector	MLS
Humidified incubator set to 37°C	MLS
Measuring cylinders (100 mL, 1 L, and 2 L)	MLS
Tubes, plates, and other consumables	
Lithium heparin blood-collection tubes, 1 tube per animal	MLS
23G–18G 1.5-inch Vacutainer needles, 1 needle per animal	MLS
Sterile, graduated 1- or 5-mL pipettes	MLS
24-well tissue-culture plate, 1 plate per 8 animals	MLS
1-mL microtubes in 96-well format with rack and caps, 1 rack per 30 animals	MLS
Polypropylene tubes	MLS
Pipette tips (as recommended by pipette manufacturer)	thermofisher.com/pipettetips
Solution reservoirs	MLS
Reagents	
Deionized or distilled water (2 L)	MLS
RPMI medium or PBS (1X), pH 7.4	MLS

[1] MLS: Fisher Scientific (fisherscientific.com) or other major laboratory supplier.
[2] Plate reader must be capable of measurement at 450 nm with a reference filter at 620–650 nm.

Procedural guidelines

- Strictly follow National Safety Regulations.
- Perform the kit protocol in laboratories that are suited for its purpose.
- Consider samples as potentially infectious and all items that contact the samples as potentially contaminated.
- Handle all primate blood samples in a biosafety cabinet (BSC, level II).
- Assay all samples and controls in duplicate.
- Dispose of containers and unused reagents in accordance with local biomedical waste requirements.
- Change pipette tips after every pipetting step.
- Maintain separate solution reservoirs for each reagent.
- Do not use kit components after their expiry date or if you observe changes in their appearance.
- Do not use kit components that have different kit lot numbers.
- Use deionized or distilled water, or water of equivalent quality.
- Use a microplate washer for all washing steps.

Before you begin

Determine the maximum plate shaker setting

If a plate shaker is used, determine the maximum setting.

1. Verify that the plate fits securely on your shaker.
2. Add 1.6 mL of water to each well of the plate, then cover with sealing foil.
3. Determine the maximum setting that you can use on your shaker without any of the water splashing onto the sealing foil.

Dilute the antigens

1. Add 160 µL of Bovine PPD to 840 µL of RPMI medium or PBS for a final assay concentration of 300 IU/mL.
2. Add 160 µL of Avian PPD to 840 µL of RPMI medium or PBS for a final assay concentration of 250 IU/mL.

Store the diluted antigens at 2–8°C for up to 1 month.

Reconstitute the controls

1. Reconstitute Positive Primate IFN-γ Control and Negative Primate IFN-γ Control in 1.0 mL of deionized or distilled water.
2. Allow the contents to sit for at least 15 minutes until dissolved.
3. Gently invert each tube 4–5 times to mix.

Store the reconstituted controls at 2–8°C for up to 3 months.

Reconstitute Conjugate-100x Concentrate

IMPORTANT! Keep Conjugate-100x Concentrate at 2–8°C always, even during reconstitution.

1. Reconstitute Conjugate-100x Concentrate in 0.5 mL deionized or distilled water.
2. Allow the contents to sit for at least 15 minutes until dissolved.
3. Gently invert the tube 4–5 times to mix, then keep at 2–8°C until use.

Store the reconstituted Conjugate-100x Concentrate at 2–8°C for up to 3 months.

Prepare Wash Buffer-1x

Note: One 96-well microplate requires 1 L of Wash Buffer-1x.

1. *(Optional)* If Wash Buffer-20x Concentrate shows a precipitate, warm the bottle in a 37°C water bath until the precipitate is dissolved.
2. Mix Wash Buffer-20x Concentrate thoroughly.
3. Combine 1 part Wash Buffer-20x Concentrate with 19 parts deionized or distilled water.
   For example, add 50 mL of Wash Buffer-20x Concentrate to 950 mL of deionized or distilled water.
4. Mix until a clear solution is obtained.

Store Wash Buffer-1x at room temperature (22±3°C) for up to 2 weeks, or at 2–8°C for up to 1 month.

Day 1—Collect whole blood samples, then incubate with the antigens

Collect whole blood samples

1. Collect at least 5 mL of blood from each animal in heparin tubes, then transfer the samples to the laboratory at room temperature.
2. Culture the blood samples within 12 hours of collection to maintain lymphocyte viability (next section).

Aliquot the blood samples, then incubate with the antigens

1. Mix each blood sample to ensure that the heparin is dissolved, according to your mixing method.
   - **Using a roller shaker**—Rock gently for 1–2 minutes.
   - **By inverting**—Gently invert each tube 10 times.
2. Transfer three, 1.5-mL aliquots of each blood sample to separate wells of a 24-well tissue-culture plate (see Table 2).

3. Add 100 µL of Nil Antigen Control (N), diluted Bovine PPD (B), or diluted Avian PPD (A) to the appropriate wells containing blood, according to the following table.

Table 2 Recommended Sample Plate layout

	1	2	3	4	5	6
A	Animal 1 N	Animal 1 B	Animal 1 A	Animal 2 N	Animal 2 B	Animal 2 A
B	Animal 3 N	Animal 3 B	Animal 3 A	Animal 4 N	Animal 4 B	Animal 4 A
C	Animal 5 N	Animal 5 B	Animal 5 A	Animal 6 N	Animal 6 B	Animal 6 A
D	Animal 7 N	Animal 7 B	Animal 7 A	Animal 8 N	Animal 8 B	Animal 8 A

4. Using a plate shaker, thoroughly mix the samples with the antigens for 1 minute (see "Determine the maximum plate shaker setting" on page 3).

   Alternatively, if a plate shaker is not available, swirl the tissue-culture plate to mix. Hold the lid and plate firmly together, swirl 10 times in a clockwise motion, then repeat in a counter-clockwise motion.

IMPORTANT! For optimal assay performance, ensure that the samples are thoroughly mixed with the antigens. It is not possible to over mix.

5. Transfer the tissue-culture plate to a humidified incubator set to 37°C, then incubate for 16–20 hours.

Day 2—Harvest plasma, then perform the ELISA

Before you begin

Equilibrate all kit components (except Conjugate-100x Concentrate) to room temperature, then gently mix reagents, samples, and controls.

Note: Some reagents can require several hours to equilibrate. If a shorter equilibration time is required, warm the reagents in a 30°C water bath.

Harvest plasma, then store samples (optional)

1. Remove the tissue-culture plate from the incubator.

2. *(Optional)* Centrifuge the plate at 500 × *g* at room temperature for 5 minutes.

3. Avoiding the bottom layer of red blood cells, carefully pipette to transfer 150–300 µL of plasma to a clean 1-mL microtube in a 96-well storage rack (see Table 3).

   Note: It is important to avoid harvesting red blood cells with the plasma during this step. However, a few red blood cells will not affect the assay.

   The following storage layout allows for efficient transfer of samples from the storage rack to the microplate strips, using a multichannel pipette.

Table 3 Recommended plasma storage layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	1N[1]	1B	1A	2N	2B	2A	3N	3B	3A	4N	4B	4A
B	5N	5B	5A	6N	6B	6A	7N	7B	7A	8N	8B	8A
C	9N	9B	9A	X	X	X	10N	10B	10A	11N	11B	11A
D	12N	12B	12A	13N	13B	13A	14N	14B	14A	15N	15B	15A
E	16N	16B	16A	17N	17B	17A	18N	18B	18A	19N	19B	19A
F	20N	20B	20A	21N	21B	21A	22N	22B	22A	23N	23B	23A
G	24N	24B	24A	X	X	X	25N	25B	25A	26N	26B	26A
H	27N	27B	27A	28N	28B	28A	29N	29B	29A	30N	30B	30A

[1] Each well indicates the animal number followed by the antigen (N—Nil Antigen Control, B—Bovine PPD, A—Avian PPD, X—empty).

Note: After the samples are transferred to the microplate strips, use the empty wells (X) for the controls that are supplied with the kit.

4. *(Optional)* Store plasma samples in sealed microtubes at 2–8°C for up to 28 days, or at −20°C for up to 5 years.

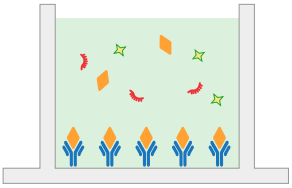
   Ensure that sample racks are dated and include the initials of the operator, tube contents, and animal information.

Perform the ELISA

- Equilibrate all kit components (except Conjugate-100x Concentrate) to room temperature, then gently mix reagents, samples, and controls.
- Determine the number of 8-well microplate strips that are required for the assay, then place the strips in the microplate frames. Return any unused strips and frames to the bag, then store at 2°C to 8°C for future use.

= Anti-IFN-γ antibody | = IFN-γ | = Conjugate | = Substrate

1 Incubate the samples with Green Diluent



a. Add 50 µL of Green Diluent to each well of the assembled microplate.

b. Transfer 50 µL of each plasma sample to the appropriate well containing Green Diluent.

   The plasma samples for each animal must be added to the wells at the same time. We recommend using a multichannel pipette.

   Note: Plasma samples can clot when thawed. Pipette carefully to ensure that the correct volume of plasma is added to each well.

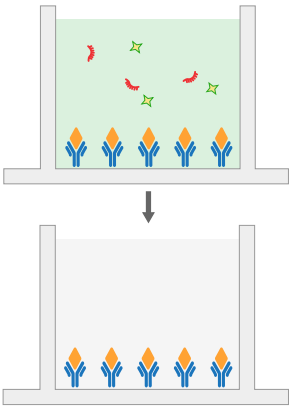
c. Add 50 µL of each control to the appropriate well containing Green Diluent.

d. Mix the samples and controls with Green Diluent using a plate shaker set at moderate-high speed for 1 minute.

   Alternatively, if a plate shaker is not available, pipet up and down 5 times to mix.

e. Cover the plate with a lid, then incubate for 60 minutes at room temperature.

2 Wash the wells



a. Wash the wells of the microplate 6 times with 400 µL of Wash Buffer-1x, allowing the wells to soak for 10 seconds between each wash.

   Note: Carefully fill the wells with Wash Buffer-1x to avoid cross-contamination with adjacent wells.

b. Tap the plate over a paper towel to remove all Wash Buffer-1x from the wells.

3 Dilute Conjugate-100x Concentrate

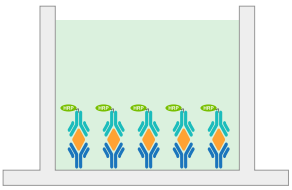
IMPORTANT! Prepare Conjugate-1x no longer than 15 minutes before use.

a. For each 8-well microplate strip, combine 10 µL of Conjugate-100x Concentrate with 1 mL of Green Diluent.

b. Mix the Conjugate-1x thoroughly. Avoid frothing, which can cause denaturation of the Conjugate.

c. Return the unused portion of Conjugate-100x Concentrate to 2–8°C storage.

4 **Add Conjugate-1x**



a. Add 100 µL of Conjugate-1x to each well of the microplate.

b. Mix thoroughly using a plate shaker set at moderate-high speed for 1 minute.

Alternatively, if a plate shaker is not available, pipet up and down 5 times to mix.

c. Cover the plate with a lid, then incubate for 60 minutes at room temperature.

d. Aspirate the solution from the wells, then wash the wells 6 times with Wash Buffer-1x (see "Wash the wells" on page 6).

5 **Prepare the enzyme substrate solution**

IMPORTANT!

- Prepare the enzyme substrate solution immediately before use.
- Use sterile, plastic polypropylene containers. Do not use polystyrene containers or pipettes.

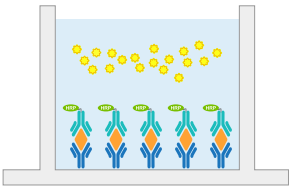
a. For each 8-well microplate strip, combine 10 µL of Chromogen Solution-100x Concentrate with 1 mL of Enzyme Substrate Buffer.

Example: For six microplate strips, combine 60 µL of Chromogen Solution-100x Concentrate with 6 mL of Enzyme Substrate Buffer.

b. Mix the enzyme substrate solution thoroughly.

Note: If blue discoloration occurs, discard the solution.

6 **Add the enzyme substrate solution**



Note: Start the 30-minute incubation time when the enzyme substrate solution is added to the first well.

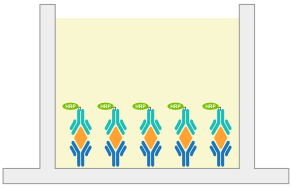
a. Add 100 µL of enzyme substrate solution to each well of the microplate.

b. Mix thoroughly using a plate shaker set at moderate-high speed for 1 minute.

Alternatively, if a plate shaker is not available, pipet up and down 5 times to mix.

c. Cover the plate with a lid, then incubate for 30 minutes at room temperature in the dark.

7 **Add Enzyme Stopping Solution, then read the plate**



a. Add 50 µL of Enzyme Stopping Solution to each well in the same order that the enzyme substrate solution was dispensed.

b. Tap the side of the plate to gently mix.

The solution in the wells changes from blue to yellow.

c. Read the plate at 450 nm (reference filter at 620–650 nm) within 5 minutes.

Analyze the results

Calculate the mean absorbance

1. Calculate the mean OD_{450} value of all samples and controls.
2. If IFN-γ is detected in Nil Antigen Control-stimulated samples, subtract the mean OD_{450} value from the results for that animal.

   Note: Detection of IFN-γ in Nil Antigen Control-stimulated samples can indicate coinfection or another disorder.

Validation criteria

If the following criteria are not met, the results are invalid and the samples must be retested.

Reaction type	mean OD_{450}	Coefficient of variation (%)
Negative Primate IFN-γ Control	<0.150	30
Positive Primate IFN-γ Control	>1.00	15

Interpretation of results

Interpret the results according to the following table:

Mean OD_{450}	Interpretation
Bovine PPD – Avian PPD > 0.050 and Bovine PPD – Nil Antigen Control > 0.050	Positive
Avian PPD > Bovine PPD and Avian PPD > Nil Antigen Control	Negative[1]

[1] The animal is considered an avian reactor.

- For absorbance readings that are outside of the limit of the plate reader, see "Retest samples with invalid results" on page 8.
- This test can give a false-positive or false-negative result due to local conditions. Interpret the results in the context of all available clinical, historical, and epidemiological information relevant to the animal being tested. Further confirmatory testing can be required in specific circumstances.
- Test interpretation and consequent animal husbandry decisions are the responsibility of the user, any consulting veterinarian, animal health advisor, or authority.

Retest samples with invalid results

1. Dilute the plasma samples 1:4 in Green Diluent.

   For example, add 25 µL of test plasma to 75 µL of Green Diluent.

2. Vortex at a moderate-high speed 3–5 times, then repeat the ELISA procedure.

Customer and technical support

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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Revision history: Pub. No. MAN0018635

Revision	Date	Description
A.0	25 November 2019	• Converted the legacy document (Primagam_PI_V1.4e_140512.pdf) to the current document template, with associated updates to the publication number, limited license information, warranty, trademarks, and logos. • Updated the procedures for antigen preparation and blood stimulation.

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