Chile 523 (1603) VILLA MARTELLI - Buenos Aires, Argentina
TELEPAX: 54-11-4761-8831/4730~4211 info@laboratorioifi.com.ar

Laboratorio ifi

- 1 -IFI FLUOR PARASITEST Toxoplasma Gondii -

FOR IN VITRO DIAGNOSTIC USE

IFIFLUOR PARASITEST SYSTEM COMPONENTS x 50 det.:

Materials provided:

The number of each component is according with the number of determinations.
Substrate slides: 10 slides of 7 wells each coated with non viable stabilized Toxoplasma Gondii. The slides design reduces the possibilities of cross-contamination of wells.

PBS buffer:

3 bags of Phosphate-Buffered Saline powder (pH 7.2 ± 0.2) each to prepare 1 liter of buffer.

Mounting medium. 5ml of glycerol buffered mounting medium. Ready to use.

Positive Control:

1 ml vial of human serum reactive for Toxoplasma Gondii, negative for HIV, HBsAg and HCV with FDA approved method. Ready to use. DO NOT DILUTE AGAIN. It contains 0.1% sodium azide.

Coverslips: 10 glass coverslips

FITC labeled antybody:

0.2 ml of goat anti-human gamma globulin labeled with fluorescein isotiocinate (FITC) concentrate .Before use it should be diluted with PBS buffer according with the vial label

Materials required but not provided:

Pipettes to deliver 20-80 ul. volumes
Coplin jars or staining dishes.
Petri dishes or another moist covered chamber
Pasteur or serological pipettes
One liter container(for PBS buffer)
Distilled water
Paper towels
Timer
Fluorescent microscope equipped with fluorescein filter.

Procedure

1. **RECONSTITTUTE BUFFER (PBS)**
   Dissolve the PBS powder in one liter of distilled water.
   The solution is stable for at least 4 weeks at 2-8C

2. **DILUTE SAMPLES**
   For Toxoplasma Gondii, samples must be diluted 1/32 with PBS. For example 100 ul serum with 3.1 ml of PBS.
   NOTE: for titering, perform double dilutions of the samples with PBS

3. **PREPARE SUBSTRATE SLIDES**
   Remove slides from the pouch. Let the slides dry at room temperature or use a hair dryer.
   Place one drop (25-40 ul) of positive and negative controls in the control wells. Place 25-40 ul of diluted sample in the remaining wells.

   CAUTION: AVOID DIRECT CONTACT OF DROPPER TIP WITH THE SLIDE SURFACE. IT MAY RESULT IN DAMAGE TO THE ANTIGEN SUBSTRATE.

4. **INCUBATE SLIDES** (20 min. at room temperature i.e. 18-25 C):
   Place slides into a moist covered chamber (a Petri dish with moistened paper toweling may be adequate) Incubate 20 min.at room temperature (18-25 C).

5. **PBS RINSE**:
   Remove slides from the incubator tray and rinse briefly with PBS using a Pasteur of serological pipette. Do not squirt buffer directly on the wells.
   NOTE: To avoid cross contamination, direct PBSS along the midline of slide among the wells, and turn the slide to one side and to another to ensure proper rinsing of every well.

6. **PBS WASH**:
   Place slides 10 min. In a Coplin jar or staining dish filled with PBSS. Agitation during the incubation is recommended. The wash may be extended without affecting the results.
   Dry carefully among the wells with a tissue towel **WITHOUT** touching the wells.
   DO NOT LET THE WELLS DRY AFTER THE PBS WASHING.

7. **FITC LABELED ANTIBODY INCUBATION**.
   (dilute with FITC labeled Ab with PBS according with the vial label).
   Put 25-40 ul of diluted FITC labeled Ab on each well. Incubate at room temperature in the moist covered chamber during 20 min.

8. **PBS RINSE AND WASH**: Repeat steps 5 and 6.

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9. **MOUNTING**:

Put one drop of mounting media on the slide and cover it with a clean coverslip. Avoid any bubbles that would difficult microscopical observation.

10. MICROSCOPICAL READING:

It is recommended to read the slides during the same day of processing.. For a longer storage we recommend to seal the coverslip with clear nail polish and storage at 2-8 C.

11.**INTERPRETATION OF RESULTS:**

Positive control must show a clear green fluorescent of the Toxoplasma Gondii observed with the microscope equipped with fluorescein filter. Negative control should not exhibit any green fluorescence.
Samples that present green fluorescent Toxoplasma images should be informed as "reactive" for Toxoplasma gondii.
If titering is required, the last positive well should indicate the titer of the sample.