



## Immunofluorescence test for the detection of *Burkholderia pseudomallei* in clinical specimen

### Lot number:

**Volume of IFA Reagent:** 150 µl (10X IFA reagent)

**Positive and Negative control:** 1 ml of killed *B. pseudomallei* and *B. thailandensis*, respectively.

**Storage condition:** Store at 2-8°C, solutions should be mixed and leaved at room temperature before use

### Expire date:

**Purpose:** To detect *B. pseudomallei* in clinical specimen

**Note:** The immunofluorescence test is based on specific reaction between monoclonal antibody (MAb) and exopolysaccharide of *B. pseudomallei*<sup>1, 2, 3</sup>.

### Method:

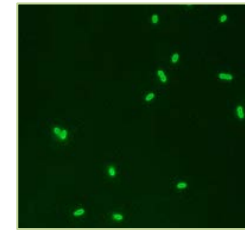
- Freshly prepare 1X IFA working reagent by diluting 10X concentrated IFA reagent to 1:10 in PBS, as follows  
Composition for 1 reaction  

10X IFA reagent	1.0	µl
PBS	9.0	µl
- Pipette 10 µl of 1X IFA reagent onto a glass slide
- Add 10 µl of sample into reagent and mix gently with a toothpick and place a coverslip
- Leave at room temperature for 10 min
- Observe the slide under fluorescent microscope using 40X and 100X immersion oil objective lenses

### Interpretation:

Negative: Not found bacteria

Positive: Found bright green fluorescent bacilli



AlexaFluor488 conjugated-MAb stained *B. pseudomallei* observed under a fluorescent microscope with 1000X magnification

### Precautions:

- False-positive result for this immunofluorescence test may occur in these following organisms *Staphylococcus aureus*. However, they are different from *B. pseudomallei* in that *S. aureus* is gram positive cocci.
- B. pseudomallei* is classified as **Category B** bioterrorism agent by CDC, creating of aerosol may occurred. This test is recommended to perform in biosafety cabinet. Glove and gown should be worn.
- Contaminated glass slide should be decontaminated with proper disinfectant before dispose in sharp container.

### References:

- Anuntagool N, et al. 2000. J Med Microbiol. 49:1075-1078.
- Tandhavanant et al. 2013. Am J Trop Med Hyg. 89(1):165-8.
- Chantratita et al. 2013. Am J Trop Med Hyg. 89(5):971-2.