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| **Intended Use** | | | |
| BZ IsoMDxTM TB/NTM kit is an in vitro diagnostic using LAMP(Loop-mediated Isothermal Amplification) assay for qualitative detection of Mycobacterium tuberculosis (MTB) and Non-tuberculous Mycobacterium (NTM) disease from DNA extracted from human sputum  **Introduction**  In 2017, about 10 million people (133 per 100,000 people) in 216 countries around the world.  It was estimated that there was a tuberculosis patient. Men, women, and children (under 15 years old) accounted for 5.8 million, 3.2 million and 1 million, respectively.  The deaths from tuberculosis were 1.6 million, of which 1.3 million were HIV (human immunodeficiency virus) negative tuberculosis (17 per 100,000), and 300,000 HIV-positive tuberculosis (21 per 100,000). In the last five years, the regions with the fastest declining average mortality rates were in WHO Europe (11% per year) and WHO East Asia (4.3% per year) and the slowest declining regions were in WHO Africa (1.7% per year).  Worldwide, the tuberculosis mortality rate is declining by 3% per year, and the tuberculosis mortality rate has decreased by 42% from 2000 to 2017.  Drug-resistant TB patients who developed in 2017 (Multidrug-resistant TB or rifampicin-resistant TB, MDR/RR-TB) is 558,000 people, and multi-drug resistance  Tuberculosis patients (MDR-TB) accounted for 82% of all drug-resistant patients.  On the other hand, non-tuberculous mycobacteria have been known as non-pathogenic bacteria commonly present in the natural environment. However, as it was identified as an opportunistic infection in patients with acquired immunodeficiency syndrome, its importance began to emerge, and recently, it has been known that it can cause infection in patients with normal immune function. According to a national survey, non-tuberculosis mycobacterial disease has been continuously increasing since the 1990s. Tuberculosis and non-tuberculosis mycobacteria commonly show symptoms such as fatigue, cough, sputum, and chest pain, which are not specific symptoms that can only be seen in patients with tuberculosis and non-TB mycobacteria. Moreover, since non-tuberculosis mycobacteria show high resistance to existing anti-tuberculosis agents, they must be treated with long-term drug combination therapy, so there is an additional need for a method that can differentiate between tuberculosis and non-tuberculosis mycobacteria.  **Principle**  BZ IsoMDxTM TB/NTM kit is developed to use the real-time multiplex LAMP method using Taqman probe. DNA extracted from patient specimen is target genes are amplified by Loop-mediated Isothermal Amplification (LAMP) using primers specific to six site at viral genome in order to detect IS6110 gene and RpoB gene simultaneously. In this process, the fluorescence signal decomposed from the fluorescence probe is detected by real-time LAMP. | | | |
| **Materials Provided (100 tests/kit)** | | | |
| |  |  |  | | --- | --- | --- | | Components | Volume | Storage | | 2X 1 step LAMP Mix  (2 EA) | Each  725 μL | Below -20℃ | | TB/NTM Primer/Probe Mix | 450 μL | Below -20℃ | | Positive control | 300 μL | Below -20℃ | | Negative control | 300 μL | Below -20℃ | | | | |
| **Materials Required but Not Provided** | | | |
| 1. Appropriate (optical) 96-well reaction PCR plate or tube 2. Micropipette 3. Centrifuge, Vortex mixer 4. Disposable powder-free gloves 5. Any of following PCR machine    1. CFX96TM Dx System (Bio-Rad Laboratories, Inc.)    2. Rotor-Gene Q (QIAGEN, Inc.)   **Warnings and Precautions**   1. For Professional Use Only 2. Be careful when handling specimens as they cannot exclude infections such as unknown microorganisms or other infectious diseases. 3. Wear lab clothing and disposable rubber gloves or vinyl gloves while handling specimen and using this product. 4. (Disposable items are prohibited to reuse.) 5. Do not chat or eat while using the product. 6. Be careful not to contaminate the specimen or product when you open the tube cap or take out the contents. 7. When processing specimen and testing with the product, filter tip should be used to prevent contamination. 8. When using this product, we recommend testing in a clean bench to prevent contamination. 9. Mixing with previous lot product is prohibited. 10. Dispense the reagents and store the reagents after freezing (below -20 ℃) for long term storage. 11. Because LAMP is a very sensitive method, take care to avoid carry-over during the test. 12. Wastes generated during the experimental should be discard in the waste container and managed according to the waste management regulations. 13. It is recommended to use the commercial RNA extraction kit. [QIAamp DNA Mini Kit(Cat. No. 51306]. 14. The final diagnosis should not be based solely on the results of this product. The final diagnosis should be based on a combination of different test methods and clinical results at the discretion of the physician. | | | |
| **Test Procedure**  **Specimen collection and handling**  It is recommended to use the lower respiratory tract specimens of people with symptoms of Mycobacterium tuberculosis or Non-tuberculous Mycobacterium(TB/NTM) infection and store them under the following conditions.  Specimen from lower respiratory tract   1. Sputum: Collect sputum into the sterilization container (sputum cans, etc.) by inducing cough to prevent saliva contamination. 2. To ensure accurate test results, immediately store the bottle containing the sample in the refrigerator (4℃) until the test. 3. To remove the inhibitory component, put 1 ml of 4% NaOH in 1 ml of the sample and leave it for 10 minutes to separate the viscous material of the sputum, and then turn it to 13,000 rpm for 10 minutes from the centrifuge and discard the supernatant. Finally, put 1 ml of PBS buffer solution and turn it back to 13,000 rpm for 10 minutes in centrifuge to discard the supernatant and extract DNA.   **DNA Sample preparation and storage**  When using the Boiling method   1. For proper DNA separation of collected sputum, CerbroSpinal Fluid (CSF), BrochioAlveolar Lavage (BAL), urine and blood samples, specimens shall be injected directly into Lysis buffer reagent without pretreatment kit. 2. To reduce the viscosity of the sputum, mix and Vortex the appropriate amount of PBS to cool the viscosity of the sputum at 95°C for 5 minutes.   When using the QIAamp® DNA mini kit,   1. Take into account the types of samples to be used and extract them according to the instructions in the kit.   **Master Mix set up**   1. Mix the components following the table below.  |  |  | | --- | --- | | Components | Volume (1 test) | | 2X 1 Step LAMP Mix | 14.5 μL | | TB/NTM Primer/Probe Mix | 4.5 μL | | Total volume of Master mixture | 19 μL |  1. Dispense 19 μL of the Master mixture into each well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube. 2. Add 6 μL of DNA sample into each well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube, and mix 2~3 times. 3. Set the PCR machine with appropriate detection channel.   **※** **Fluorescent Reporter**   |  |  | | --- | --- | | Detection target | Reporter | | IS6110 gene | Cy5 | | RpoB gene | FAM | | Internal Control GAPDH (IC) | HEX |  1. Perform PCR amplification step as follows. (Do not set up the passive reference).  |  |  | | --- | --- | | **Step** | **Amplification** | | Temperature | 62°C | | Time | 1 min | | Cycle | 40 |   **Data Analysis**   1. Analysis setting 2. Set the baseline of all PCR results using flat signal in an initiation phase. 3. Set up the threshold by PCR system as follows.  |  |  | | --- | --- | | Instrument | Threshold | | CFX96TM Dx system | 500 (RFU) | | Rotor-Gene Q | 0.1(RFU) |  1. Acceptance Criteria 2. Positive: Ct value of signal is 35 or less. 3. Negative: Ct value is not detected. 4. Interpretation of Results  (Examples of Positive/Negative result)  |  |  |  |  |  | | --- | --- | --- | --- | --- | | No. | Cy5 (IS6110) | FAM (rpoB) | HEX  (GAPDH) | Results interpretation | | 1 | Positive | Positive | Positive | TB Positive | | 2 | Positive | Positive | Negative | | 3 | Positive | Negative | Positive | | 4 | Positive | Negative | Negative | | 5 | Negative | Positive | Positive | NTM Positive | | 6 | Negative | Positive | Negative | | 7 | Negative | Negative | Positive | Negative | | 8 | Negative | Negative | Negative | Invalid |   ※ Even if the internal control is negative, it is positive if the target fluorescence is positive.  ※ In the case of the test results are positive, even of the results of the test are strong and the internal control is not shown, it should be determined as positive.  ※ The test results of both negative and positive controls should be valid. If either one is not valid, retest.  **Performance Characteristic**  **Analytical sensitivity (Limit of Detection)**  To determine the analytical sensitivity of BZ IsoMDxTM TB/NTM kit, the lower respiratory tract specimens (Sputum) were diluted with internal standard material, and was tested 20 times. The concentration of 100% or more positive result was determined as the minimum detection limit.  The limit of detection is 103 copies/µL for the specimens from the lower respiratory tract regardless of the PCR systems including CFX96TM Dx System (Bio-Rad)..  **Analytical sensitivity (Cut off Value)**  The cut off value was determined as **35** based on the Ct value, which was set using the LOD (Limit of detection) test result value.  **Analytical specificity (Cross Reactivity)**  To evaluate the cross reactivity of BZ IsoMDxTM TB/NTM kit, the possible cross reactive pathogens as listed in the table below were tested 3 repeated times.  As a result, no cross reactivity was observed for the pathogens showing the similar symptoms or alpha coronavirus.  *- Human Astrovirus (RNA)*  *- SARS CoV (RNA)*  *- Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (RNA)*  *- Haemophilus influenza*  *- Adenovirus (DNA)*  *- Mycoplasma pneumoniae (DNA)*  *- Human metapneumovirus (DNA)*  *- Human Bocavirus (HBoV) (DNA)*  *- Staphylococcus aureus strain (MRSA) (DNA)*  *- Enterobacter cloacae*  *- Legionella pneumophila (L.Pn)*  *- Nippostrongylus brasiliensis*  *- Methicillin Resistant Staphylococcus (MRS A)*  *- Methicillin Resistant Staphylococcus (MSS A)*  *- Streptococcus pyogenes*  *- Streptococcus pneumoniae (S.pn)*  *- Staphylococcus aureus*  *- Klebisiella Oxytoca (K. Oxytoca)*  *- Klebsiella pnrumoniae (K.pn)*  *- Pseudomonas aeruginosa*  **Analytical specificity (Interference)**  To test the effect of the possible interfering substances BZ IsoMDxTM TB/NTM kit was tested 3 repeated times using specimen prepared by adding the materials listed below. (Mucin 1%, Acetyl salicylic Acid 15mg/mL, NaCl 7.4mg/mL, Oxymetazoline 20%, Hemoglobin 0.2%, Whole blood 3%)  **Precision (Reproducibility)**  To evaluate reproducibility of BZ IsoMDxTM TB/NTM kit for sputum from the lower respiratory tract, two runs of test were performed each day. Each test was repeated twice with 1 lot by two experimenters in 3 different places for 5 days.  As a result, the precision between places and between experimenters showed 100% consistency for each sample. SD and CV are below 0.4 and 2.31 in sputum.  **Precision (Repeatability)**  To evaluate repeatability of BZ IsoMDxTM TB/NTM kit for sputum from the lower respiratory tract, two runs of test were performed each day. Each test was repeated twice with 3 lots for 20 days. Specimens used includes strong positive sample(3×LOD), weak positive sample(1×LOD) and negative sample.  As a result, the precision by day and lot was 100% consistent for each sample. SD and CV are below 2.2 and 7.99 in sputum.  **Storage condition**  BZ IsoMDxTM TB/NTM kit components: Store below -20℃ (sealed). It is stable and can be used for 6 months from the date of manufacture.  **Reference**  1. World Health Organization (WHO). Diagnostics for tuberculosis: global demand and market potential, Geneva : WHO on behalf of the Special Programme for Research and Training in Tropical Diseases , 2006.  2. P W Wright & R J Jr Wallace & N W Wright & B A Brown & D E Griffith, Sensitivity of fluorochrome microscopy for detection of Mycobacterium tuberculosis versus nontuberculous mycobacteria, 1998, *J Clin Microbiol*, 36:1046.  3. Marras Theodore K & Daley Charles L, Epidemiology of human pulmonary infection with mycobacteria nontuberculous, *Clin Chest Med* 2002, 23:553.  4. Wagner D & Young LS, Nontuberculous mycobacterial infections: a clinical review, Infection - A Journal of Infectious Disease 2004, *Infection*, 32:257. | | | |
| **Description of Symbol Used**   |  |  |  |  | | --- | --- | --- | --- | | Symbol | Description | Symbol | Description | | 설명: C:\Users\ACCESSBIO18\Desktop\TO DO\IMAGES!!!!!!!!!!!!!!\Catalogue number.PNG | Catalogue number | 설명: C:\Users\ACCESSBIO18\Desktop\TO DO\IMAGES!!!!!!!!!!!!!!\Caution.PNG | Caution | | 설명: C:\Users\ACCESSBIO18\Desktop\TO DO\IMAGES!!!!!!!!!!!!!!\Batch code.PNG | Batch code | 설명: C:\Users\ACCESSBIO18\Desktop\TO DO\IMAGES!!!!!!!!!!!!!!\Manufacturer.PNG | Manufacturer | | 설명: C:\Users\ACCESSBIO18\Desktop\TO DO\IMAGES!!!!!!!!!!!!!!\Use-by date.PNG | Use-by date | 설명: C:\Users\ACCESSBIO18\Desktop\TO DO\IMAGES!!!!!!!!!!!!!!\Consult instructions for use.PNG | Consult instructions for use | | 설명: C:\Users\ACCESSBIO18\Desktop\TO DO\IMAGES!!!!!!!!!!!!!!\Temperature Limit symbol.PNG | Upper limit of  temperature |  |  | | | | |
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