

IVD

Approval no. 21700AMZ00801000

Please read this package insert carefully before use.

Reagent to Detect

M. tuberculosis Complex

Capilia TB-Neo

- Since cultures containing acid-fast bacteria and so forth are employed for the tests using this kit, the operation described below must be done in a biological safety cabinet, and used test plates must be handled with due precautions as they may cause infections.
- The test plate should be used immediately after opening the packaging.
- When it absorbs moisture, the quality deteriorates and an accurate result cannot be obtained.
- Usage, dosage and instructions should be followed when using the kit.
- Any clinical diagnosis must be made by the attending physician, taking into account all the clinical information and test results obtained with this test kit.

Incoming Quality Control: Testing of positive and negative samples should be done immediately after receipt of the Capilia Tests. This allows for checking whether or not shipment conditions have damaged the Capilia tests, resulting in decreased sensitivity. These positive and negative controls have to be repeated before each daily run.

A positive control can be:

- 1) proven clinical isolate of *M.tb* or *M.tb* H37RV or
- 2) A liquid prepared by suspending 1 μ L (equivalent to the amount of a 1mm-diameter platinum micro-loop) of a colony of 5 *Mycobacterium bovis* BCG* grown on an Ogawa medium in 0.2mL of 10mmol/L phosphate-buffered saline (PBS) containing 0.1%(w/v) Tween 80, or
- 3) A liquid culture of *Mycobacterium bovis* BCG* (equivalent to a McFarland No.1 standard (3 to 6 x 10⁷CFU/mL)) grown on an AFB liquid medium.

A negative control is:

- 1) Non-inoculated liquid media and
- 2) 10mmol/L phosphate-buffered saline (PBS) containing 0.1% (w/v) Tween 80, and
- 3) Any non-tuberculous *Mycobacteria* such as *M. smegmatis* or *M. goodii*

* Please refer to following information about BCG strains as a positive control. Some BCG strains, Glaxo strain, Pasteur strain, Tice strain, do not express MPB64. Therefore only Brazilian, Japanese (Tokyo), Russian, Swedish ones are used as a positive control of a BCG strain. Please refer to the following publication, *Journal of Clinical Microbiology*, Nov. 1999, p3693.

1. Background of the development and its features

(1) Background of the development

Tuberculosis (TB) is a chronic disease caused by infection with *Mycobacterium tuberculosis*. The number of new TB cases and deaths from TB is estimated at around 8.5 millions (as of 2001) and 1.8 millions (as of 2000), respectively. Ninety-five per cent of the estimated incidence occurred in developing countries and the incidence in Asia, Africa, Middle East and Latin America amounts to 5 millions, 2 millions, 0.6 millions and 0.4 millions, respectively. Also in developed countries, the pace of reduction in TB incidence has been dropping since the 1980s due to (i) the increased number of elderly and HIV-infected people whose immunities have deteriorated, (ii) the increased number of immigrants from countries highly infested with TB, (iii) the emergence of a multi drug-resistant *M. tuberculosis* and (iv) social problems such as poverty, increase in the number of homeless people and drug abuse¹⁾²⁾.

In Japan, the number of TB incidences and deaths from the disease in 2003 was 31, 638 and 2,336, respectively. TB is still considered a serious disease³⁾.

In addition, the number of infections with non-tuberculosis mycobacterium (NTM) has been increasing in recent years.

An NTM infection may manifest the same symptoms as those of TB. Because many NTMs are resistant to ordinary antituberculous drugs, it is critical to distinguish NTM infections from TB to determine treatment.⁴⁾⁵⁾

Definite diagnosis of TB is made by detecting *M. tuberculosis* from a clinical sample. Conventionally, the following method is widely used for the detection: ① Treat a sample [body fluid (mainly sputum), organ, tissue and bronchial lavage fluid] with sodium hydroxide etc. and inoculate the sample in a medium such as Ogawa Culture for culturing acid-fast bacteria (AFB). ② Observe generated colonies of the isolate and conduct tests, including the Niacin accumulation test¹⁾, to determine the biochemical property of the isolate. ③ Identify and differentiate TB and NTM from the observations and the results of the above-mentioned tests.⁶⁾⁷⁾ However, it usually takes 4 to 8 weeks to obtain such test results since the growth of acid-fast bacterium is very slow and a Niacin accumulation test requires a sufficient amount of bacterial isolate.⁶⁾

Recently, a nucleic acid probe-based identification method has been developed and test laboratories have adopted this new method as a tool for rapid detection of an *M. tuberculosis* complex. Now the identification of TB or NTM is possible with only a small amount of bacterial isolate.⁷⁾⁸⁾⁹⁾ In addition, a nucleic acid amplification method is available, which can identify an *M.tuberculosis* complex directly from clinical samples without an isolation culture.⁷⁾¹⁰⁾¹¹⁾

The new testing method, however, involves cumbersome handling procedure and requires special instruments/equipment and a skilled operator.

Under such circumstances, a new reagent that can detect an *M. tuberculosis* complex specifically and rapidly has been long awaited.

The Capilia TB-Neo kit adopts an immunochromatography method, which can detect the MPB64 antigens specifically produced by an *M. tuberculosis* complex. Thanks to this method, the kit is able

¹⁾ Niacin (nicotinic acid) functions as a precursor in the biosynthesis of the coenzymes NAD and NADP.

Although all mycobacteria produce nicotinic acid, comparative studies have shown that because of a blocked metabolic pathway for *M. tuberculosis* accumulates niacin and excretes it into the culture medium from which it can be extracted. The niacin then is detected by its reaction with a cyanogen halide in the presence of primary amine.

not only to detect an *M.tuberculosis* complex in bacterial isolates specifically with high sensitivity but also perform rapid tests with only a simple operation without special instruments and equipment^{18)–21)}.

(2) About MPB64

This product is intended to detect MPB64 (mycobacterial protein fraction from BCG of Rm 0.64 in electrophoresis), a protein secreted from the cells during the culture of an *M.tuberculosis* complex. Acid-fast bacterium is known to produce 200 or more kinds of protein. MPB64 was first isolated by Nagai et al. from the culture filtrate of *M.bovis* BCG which is an attenuated *M.bovis* strain. In 1986, Harboe et al. reported the biochemical property of MPB64 as a protein having a molecular weight of 24,000.¹²⁾

Later, a gene-based analysis confirmed that MPB64 was identical to the MPT64 produced by *M.tuberculosis*¹³⁾ and it was reported that non-tuberculosis mycobacterium (NTM) produces no MPB64 and that MPB64 is a protein specifically secreted by an *M.tuberculosis* complex (*M.tuberculosis*, *M.bovis*^(Note), *M. africanum* and *M. microti*).^{14) 15) 16)}

(Note) Four of the strains among *M.bovis* BCG – Tokyo, Moreau, Russia and Sweden – were reported to produce MPB64.

(3) Testing method

Immunochromatography is a kind of double-antibody sandwich technique, in which (i) an antibody labeled by colloidal particles such as colloidal gold reacts with target antigens to form an antigen-antibody complex, (ii) this complex migrates across a chromatographic carrier such as a filter paper and (iii) the complex is captured by a second antibody ready-fixed in the middle of the chromatographic carrier. If the target antigens are present in the test specimen, a color reaction caused by the labeled colloidal particles such as colloidal gold is observed at the site on the chromatographic carrier where the second antibody is fixed, and the specimen is interpreted as positive. This kit employs the colloidal gold-labeled MPB64 monoclonal antibody (mouse) in the main reaction system. The results are able to be visually identified as a specific antigen-antibody reaction between the monoclonal antibody and MPB64 antigens that were secreted into the isolate of the clinical test specimen.

(4) Features of the product

This product provides not only the same sensitivity and specificity as those provided by the nucleic acid probe-based detection method but also the following advantages:

1. It does not require any special equipment.
2. It is easy to operate and does not require special skills.
3. Test results can be obtained in a short time (15 min).

In a Niacin accumulation test, there is a possibility of overlooking the presence of the *M.tuberculosis* complex because the test is unable to detect the complex from mixed cultures containing NTM. In contrast, this product is able to detect the *M.tuberculosis* complex.¹⁷⁾

However, if the growth of the *M.tuberculosis* complex is slight and the MPB64 concentration in the test specimen is below the detection limit, the complex may not be detected with this product.

2. Substances (kit components)

Test plate

- Formula, quantities (per one test)
 - Colloidal gold-labeled anti-MPB64 monoclonal antibody (mouse)
 - Anti-MPB64 monoclonal antibody (mouse)

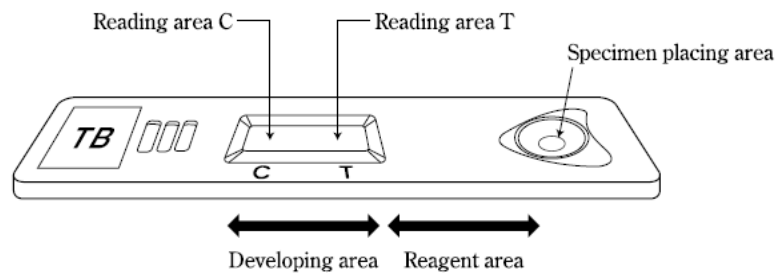
3. Indication, Effect (Intended use)

To detect an *M.tuberculosis* complex

4. Test procedure (principles of the procedure)

This product is a test plate that consists of a carrier strip composed of (i) a specimen placing area, (ii) a reagent area containing a colloidal gold-labeled anti-MPB64 monoclonal antibody (mouse) and (iii) a developing area where the anti-MPB64 monoclonal antibody (mouse) and an anti-mouse immunoglobulin polyclonal antibody (rabbit) are fixed.

Names of each area of the test plate



When a specimen is dripped on the specimen placing area of the test plate, the colloidal gold labeled MPB64 antibody A dissolves and forms an immune complex with MPB64 antigens in the specimen. This immune complex migrates through the developing area by capillary action and is captured by the anti-MPB64 antibody B (fixed antibody B) fixed in the reading area [T]. The resultant complex forms a purple red line of colloidal gold in the reading area [T]. This kit visually indicates the purple red line that shows the existence of MPB64 antigens in the specimen.

On the other hand, whether or not MPB64 antigens exist in the specimen, excess colloidal gold-labeled anti-MPB64 antibodies further migrate through the developing area and are captured by the anti-mouse immunoglobulin antibody (fixed antibody). The resultant complex forms a purple red line of colloidal gold in the reading area [C]. This means that the colloidal gold-labeled anti-MPB64 antibodies have migrated normally.

$\text{MPB64} + \text{Colloidal gold-labeled anti-MPB64 antibody} \Rightarrow \text{Complex of colloidal gold-labeled anti-MPB64 antibody and MPB64 antigens}$

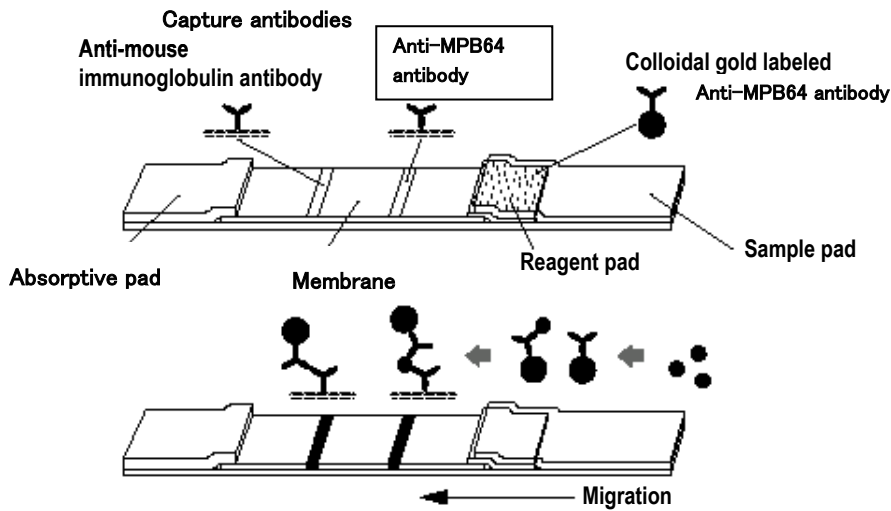
(Reaction in the reading area [T])

$\text{Complex of colloidal gold-labeled anti-MPB64 antibody and MPB64 antigens} + \text{Fixed antibody} \Rightarrow \text{Complex of colloidal gold-labeled anti-MPB64 antibody, MPB64 antigens and fixed antibody} : \text{Purple red line}$

(Reaction in the reading area [C])

$\text{Colloidal gold-labeled anti-MPB64 antibody} + \text{Fixed antibody} \Rightarrow \text{Complex of colloidal gold-labeled anti-MPB64 antibody and Fixed antibody} : \text{Purple red line}$

Detection of MPB64 using the immunochromatography



5. Precautions concerning operation

(1) Characteristics of specimen and specimen collection method

- ① A normal reaction might not occur if the amount of the specimen is not enough. It is essential to follow the usage and dosage (as well as the operation procedure) specified in the package insert.
- ② Use suspended cultures or liquid cultures inoculated in the media for AFB as specimens. No clinical samples such as a human body fluid, tissue and bronchial lavage fluid can be directly used as they are as specimens.
- ③ Since *M. tuberculosis* is a pathogen classified as a “3a hazardous mycobacterium” in the Safety Code of the National Institute for Infectious Diseases in Japan, any testing laboratory that handles *M. tuberculosis* must be isolated from the external space by dual doors and the mycobacterium must be handled in a biological safety cabinet.
- ④ Bacteria inoculated in the media for AFB must be carefully handled as they may cause infections.
- ⑤ Dispense samples with a micro-pipette and use a new tip with a filter for dispensing each specimen.

(2) Interfering Substances

Clinical tests have been carried out for this product using cultures from sputum, bronchial lavage fluid, hydrothorax, gastric juice and purulent fluid as specimens, and no interference to the test results by clinical samples has so far been observed. In addition, we have used the following media for AFB, and no interference in the test results by the media was observed:

- Egg-based media: 3% Ogawa media, 2% Ogawa media, 1% Ogawa media, Loewenstein-Jensen (LJ) media
- Agar media: Middle Brook7H10 agar media, Middle Brook7H11 agar media
- Liquid media: Middle Brook7H9 liquid media, Dubo’s liquid media, Kirchner media, Sauton’s media

As explained above, no impact from different kinds of clinical samples or culture media has so far been found in the study. However, it is not known whether or not any other substances present in specimens may affect test results.

6. Usage, dosage (Procedures)

(1) Specimen preparation

After completing appropriate pretreatment^(*) of clinical samples such as a human body fluid, tissue and bronchial lavage fluid, inoculate the sample in a culture medium for AFB.

(*) Example of pretreatment – sputum:

- N-acetyl-L-cystine-sodium hydroxide (NALC-NaOH) method:
Add at least 2-fold volume of NALC-NaOH solution to the container containing the collected sputum, stir the container with a vortex mixer and invert the container to expose the inside of the screw cap and the inner wall of the tube to NALC-NaOH solution. Let the container stand at room temperature for 15min and intermittently shake it lightly by hand. After adding cold-sterilized, 10-fold volume of phosphate-buffered (PB) solution (pH6.8) to the container and mixing it, centrifuge 3,000 x g for 20min. Suspend the precipitate in 1mL of PB solution. Then, inoculate 0.1mL of the suspension in a 1% Ogawa medium or inoculate 0.5mL of the same into a liquid medium.
 - 4% sodium hydroxide method:
Add at least 2-fold volume of 4% sodium hydroxide solution to the container containing the collected sputum, fully homogenize the mixture and immediately inoculate 0.1mL of the mixture in a 3% Ogawa medium.
- ① In the case of using liquid medium for AFB (Example: Middle Brook 7H9 broth)
Incubate at 37°C for 1 to 3 weeks until the liquid medium gets cloudy by the growth of bacteria. In the event that MGIT is used, incubate until a positive interpretation is possible. In both cases, it is necessary to confirm the presence of AFB by acid-fast staining. Stir the liquid media in the incubator and use the media as a specimen.
 - ② In the case of using a solid medium for AFB (Example: Ogawa medium)
Incubate at 37°C for 2 to 4 weeks until the growth of bacterial colonies is confirmed on the solid medium, and then confirm the presence of AFB by acid-fast staining.
 1. Dispense 0.2mL of extraction buffer^(*) into a tube. ^(*) Separately sold.
 2. Pick 1 μ L of bacteria (equivalent to the amount of a 1mm-diameter platinum micro-loop) from the bacterial colony that has grown on the solid medium
 3. Suspend the collected bacteria in the buffer solution in the tube.
 4. Close the tube with a stopper and fully suspend with a vortex mixer. Then, use the bacterial suspension as specimens.

(2) Test procedure

1. Drip an 80~100 μ L specimen into the specimen placing area of the test plate.
2. Observe the reading area of the test plate after 15 min and interpret the result as follows.
3. Interpretation:
 - Positive, if a purple red line is observed in the reading areas of both [T] and [C]
 - Negative, if a purple red line is not observed in the reading area [T] but the color is observed in the reading area [C]

Test results must be interpreted within 60 min after placing each specimen on the test plate because the results may change for some plates due to dryness etc occurring after a lapse of time.

7. Interpretation of the Results

(1) The Method for Assessing Determination Results

A reaction is produced according to the operation procedure and the test results are visually interpreted by lines appearing in the reading areas. (The reading area must be viewed from directly above.)

Positive: When lines are seen at both [T] and [C] in the reading area, the result is interpreted as positive. (*) Even if the line at [T] in the reading area is lighter than that at [C] in the reading area, the result is interpreted as positive.

Negative: When no line is seen at [T] in the reading area but seen at [C], the result is interpreted as negative.

Retesting: When no line is seen at [C] in the reading area, there is a possibility of operational problems or deterioration in the quality of the reagent used. Therefore, retesting is required using another test plate.

(2) Precautions when interpreting test results

- ① If no line is seen at [C] in the reading area, there is a possibility of operational problems or deterioration in the quality of the reagent used. Therefore, retesting is required using another test plate.
- ② Test results must be interpreted within 60 min after placing each specimen on the test plate because the results may change for some plates due to dryness etc occurring after a lapse of time. In such a case, retesting is required using another test plate.
- ③ If a test result using this kit is interpreted as positive, the presence of *M.tuberculosis* in the specimen is strongly suggested. However, there is a possibility of combined infections of *M.tuberculosis* and non-tuberculous acid-fast bacteria, and an infection with non-tuberculous acid-fast bacteria is not excludable.
- ④ In the case of protein A-producing strains such as *Staphylococcus aureus*, false positive reactions may occur. Take due precautions for such a case.
- ⑤ Even if a test result using this kit is interpreted as negative, this may be because it is unable to detect an *M. tuberculosis* complex when the MPB64 concentration in the specimen is below the detection limit or a mutation arises in the MPB 64 gene of *M. tuberculosis* complex²²⁾. Therefore, a negative result does not necessarily rule out the possibility of infection with *M. tuberculosis*.

Any clinical diagnosis must be made by an attending physician, utilizing all the clinical information and test results obtained with this test kit.

8. Performance

(1) Performance

① Sensitivity test

When a positive control is tested as a sample, positivity is confirmed.

② Specificity test

When a positive control and a negative control are tested as samples, positivity is confirmed by the positive control and negativity is confirmed by the negative control.

③ Reproducibility test

When a positive control and a negative control are tested as samples, each of which is tested three times simultaneously, positivity is confirmed by all of the positive controls and negativity is confirmed by all of the negative controls.

Positive control: A suspension in which 1 μ L of the colony of *Mycobacterium bovis* BCG-Tokyo that has been grown on 1% Ogawa's medium is suspended in 0.2 mL of 10 mmol/L phosphate-buffered physiological saline containing 0.1% (w/v) Tween 80 followed by dilution 25 times with 10 mmol/L phosphate-buffered physiological saline containing 0.1% (w/v) Tween 80; or a cultured solution diluted 25 times with a liquid culture medium for acid-fast bacilli without inoculation, wherein the cultured solution contained *Mycobacterium bovis* BCG-Tokyo grown in a liquid culture medium for acid-fast bacilli and was equivalent to McFarland No. 1 (3×10^7 to 6×10^7 CFU/mL) before the dilution.

Negative control: 10 mmol/L phosphate-buffered physiological saline containing 0.1% (w/v) Tween 80 or a liquid culture medium for acid-fast bacilli without inoculation.

④ Minimum detection limit

The minimum detection limit is 1.2×10^6 CFU/mL.

(2) The result of the cross-reactivity test

The reactivity with the following nontuberculous mycobacteria was checked and an absence of crossing has been confirmed with all of them.

M.kansaii, *M.simiae*, *M.asiaticum*, *M.scrofulaceum*, *M.gordonae*, *M.avium*, *M.intracellulare*, *M.nonchromogenicum*, *M.szulgai*, *M.terrae*, *M.xenopi*, *M.ulcerans*, *M.fortuitum*, *M.chelonae*, *M.abscessus*, *M.smegmatis*, *M.vaccae*, *M.flavescens*, *M.marinum* JATA22-01, *M.marinum* 351-2, *M.marinum* 329, *M. marinum* 60

(3) Reactivity with Standard Strains of *Mycobacterium tuberculosis* Complex

① Strains demonstrating the production of MPB64

A reaction was observed with the following 8 strains.

M.tuberculosis H37Rv, *M.tuberculosis* H37Ra, *M.africanum*, *M.bovis deer*, *M.microti*, *M.bovis* BCC-Tokyo, *M.bovis* BCG-Russia, *M.bovis* BCG-Moreau

② Strains demonstrating no production of MPB64

No reaction was observed with the following 2 strains.

M.bovis BCG-Pasteur, *M.bovis* BCG-Tice

9. Correlation with the conventional test methods

(1) WHO Strains of *M. tuberculosis*

| | | Control kit (Capilia TB) | | |
|----------|----------|--------------------------|----------|-------|
| | | Positive | Negative | Total |
| This kit | Positive | 70 | 0 | 70 |
| | Negative | 0 | 0 | 0 |
| | Total | 70 | 0 | 70 |

Concordance rate: 100% (70/70)

Sensitivity: 100% (70/70)

Specificity: — (0/0)

As the above data show, positive results were also obtained with this kit for all of the 70 WHO strains of *M. tuberculosis* for which positive results were obtained with the Control kit.

(2) Clinical Isolate of *M. tuberculosis*

| | | Control kit (Capilia TB) | | |
|----------|----------|--------------------------|----------|-------|
| | | Positive | Negative | Total |
| This kit | Positive | 46 | 0 | 46 |
| | Negative | 0 | 5* | 5 |
| | Total | 46 | 5 | 51 |

Concordance rate: 100% (51/51)
Sensitivity: 100% (46/46)
Specificity: 100% (5/5)

As the above data show, positive results were also obtained with this kit for all of the 46 clinical isolates of *M. tuberculosis* for which positive results were obtained with the Control kit.

* A gene analysis for five clinical isolates of *M. tuberculosis* for which negative results were obtained by the control kit (Capilia TB) as well as by this kit revealed that there was mutation in the base sequence of the MPB64 gene so that those isolates were mutants in which the expression of the MPB64 protein was incomplete.

10. Precautions when using and handling

(1) Precautions for handling specimens

- ① Use suspended cultures or liquid cultures inoculated in the media for AFB as specimens. No clinical samples such as a human body fluid, tissue and bronchial lavage fluid can be directly used as specimens as they are.
- ② Cultured specimens must be used immediately for testing.²
- ③ Bacteria inoculated in the media for AFB must be carefully handled as they may cause infections.
- ④ Some specimens including substrains of *Mycobacterium bovis* BCG among the *M.tuberculosis* complex (Copenhagen, Glaxo, Pasteur and Tice) are interpreted as negative since no MPB64 is produced from these substrains.
- ⑤ This kit is unable to differentiate each of the *M. tuberculosis*, *M.bovis*, *M. africanum* and *M. microti* from the others even if the *M. tuberculosis* complex test results are positive.

(2) Precautions when using

- ① This product should be used in accordance with the procedure stated in the package insert.
- ② In order to prevent deterioration, the products should be stored between 2–30°C, avoiding high temperatures, high humidity and direct sunlight.
- ③ The aluminum pouches containing test plates should not be opened until they are about to be used.
- ④ The specimen placing area or the reading area of the test plate should not be touched with the hands.
- ⑤ Dispense samples with a micro-pipette and use a new tip with a filter for dispensing each sample.

(3) Precautions for disposal

² Positive cultures in liquid media and colonies on solid media can be tested up to one year when stored at -20 °C or 2 to 8 °C for testing²⁰.

- ① Since test plates, test chips, remaining specimens and other test materials/devices used for identification of *M. tuberculosis* may cause infections, they should be autoclaved (at 121°C, over 30 min) before disposal.
- ② When sterilized used reagents or other test materials are disposed of, they should be treated in accordance with applicable laws and regulations and must be disposed of as medical wastes or industrial wastes.

11. Storage, Validity

Storage: store at 2–30°C without opening the aluminum pouches containing the test plates
Expiry: 12 months

12. Packaging Unit

For 80 tests
Extraction buffer 20mL

13. References

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Marketing Approval Holder in Japan

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