

RiboNAT™ Rapid Sterility Test

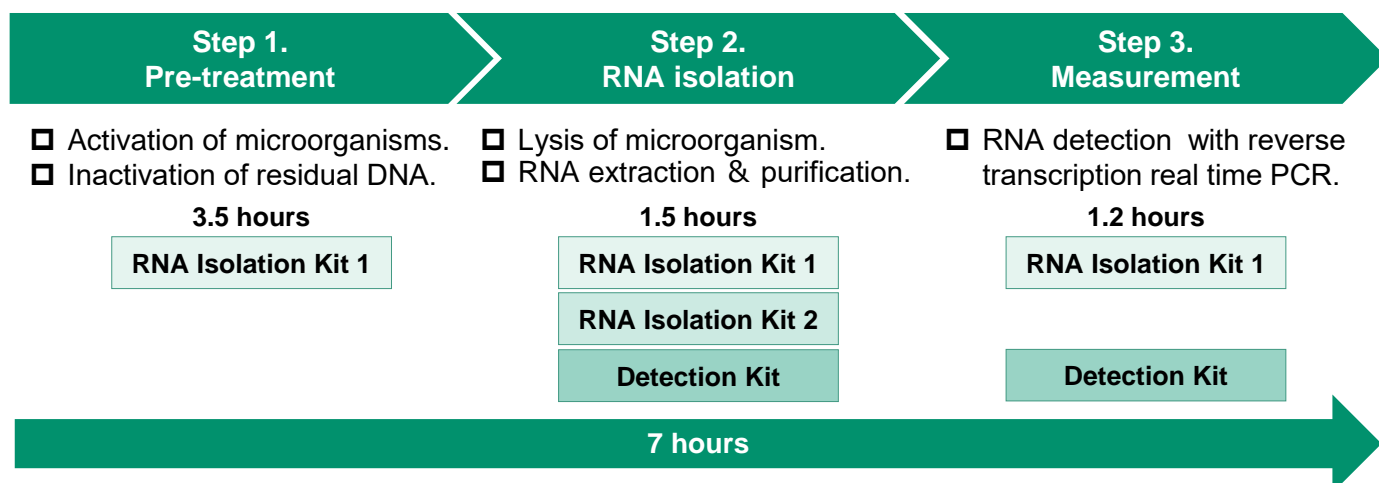
In the safety testing of parenteral drugs, the compendial sterility tests require a 14-day cultivation period. However, certain types of cell therapies must be administered to patients within just a few days after production, making it difficult to complete sterility testing before administration. This has led to a growing demand for sterility tests that yield results more rapidly to enhance patient safety.

RiboNAT™ is a rapid sterility testing kit utilizing the Nucleic Acid Amplification Test (NAT) method. It delivers results in as little as seven hours, significantly reducing testing time compared to conventional sterility testing methods. Unlike standard NAT methods, which detect genomic DNA, RiboNAT™ targets ribosomal RNA (rRNA). Because rRNA exists in higher relative quantities within microorganisms, this approach allows for more sensitive detection. Additionally, while conventional NAT methods can produce false positives due to residual DNA derived from dead microorganisms and contaminated DNA from materials and environment, RiboNAT™ minimizes this risk by inactivating dead microorganisms' DNA with Nucleic Acid Inactivator and degrading DNA with DNase.

<Features>

- One day rapid sterility test (Approx. 7 hours).
- Detecting rRNA using RT-rt PCR and higher sensitivity than gDNA detection (9 CFU/mL).
- Reducing false positives from dead microorganisms and residual DNA.
- Wide detection range of bacteria (aerobic & anaerobic) and fungi in a single assay.

<Assay Flow>



<Specifications of Detection Kit>

| | |
|------------------|---|
| Detection method | One step reverse transcription real time PCR with fluorescence probe (RT-rt PCR) |
| Sensitivity | 100 RNA copies per reaction |
| Coverage* | Bacteria: 25,748 (95.7%) Fungi: 1,683 (92.3%) *In silico analysis, Accepting 3 mismatches, Data bank: Silva |
| Target | Bacteria: 23S Ribosomal RNA (Detection wavelength: 515-530nm) Fungi: 25/28S Ribosomal RNA (Detection wavelength: 675-690 nm) Internal Control: Artifact sequence (Detection wavelength: 560-580 nm) |

<Test Sensitivity>

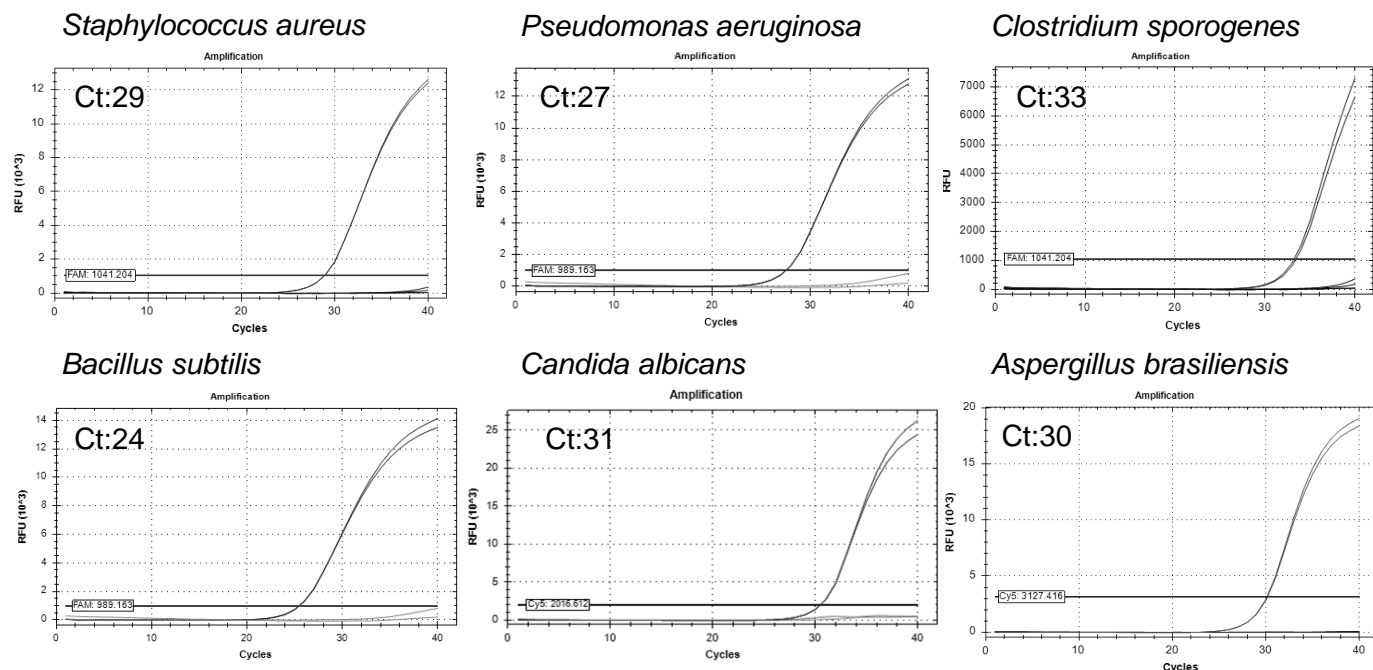


Figure 1. Test sensitivity

Prepared microbial suspensions at 9 CFU/mL for the six microorganisms specified in the pharmacopeia. RNA was extracted from these suspensions using RiboNAT™, and ribosomal RNA (rRNA) was detected according to the protocol. rRNA from all microorganisms was successfully detected with Ct values below 35.

Detectable at 9 CFU/mL

<Test sensitivity in cell suspension sample>

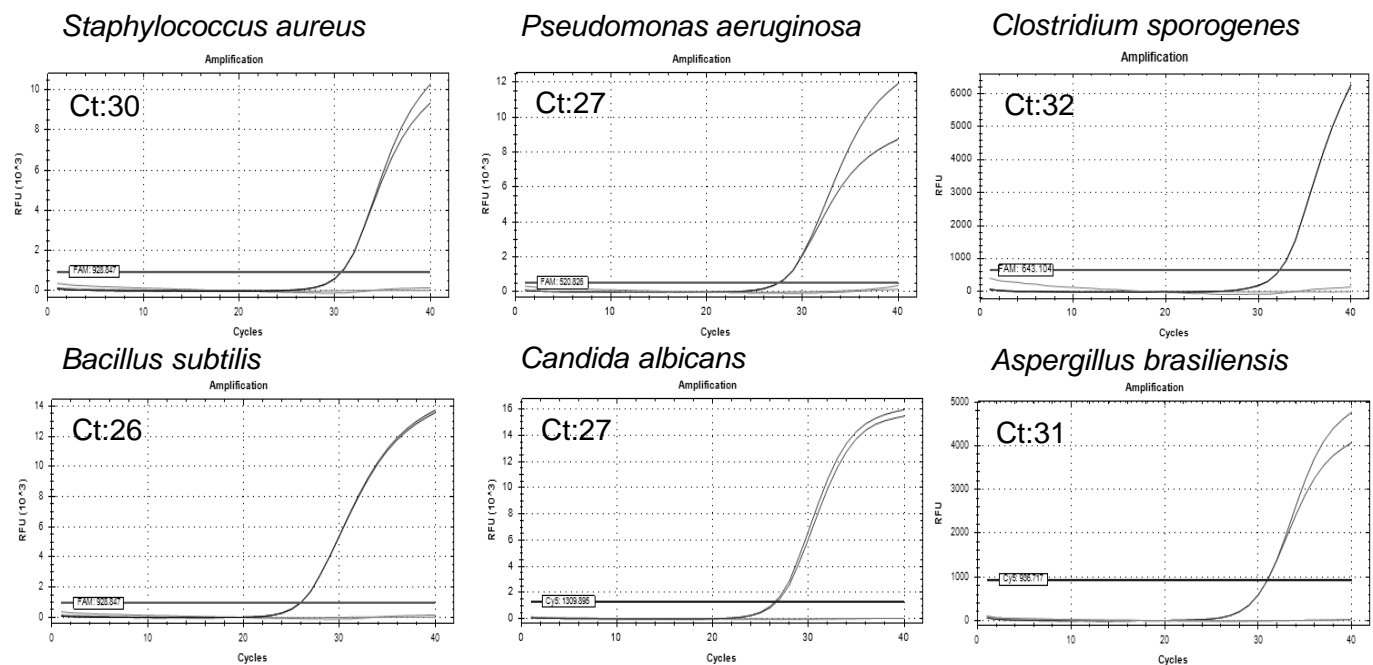


Figure 2. Test sensitivity in cell suspension sample

Prepared microbial suspensions at 9 CFU/mL for the six microorganisms specified in the pharmacopeia, each mixed with 0.25 x 10⁶ cells/mL of HEK293. RNA was extracted from these suspensions using RiboNAT™, and rRNA was detected according to the protocol. rRNA from all microorganisms was successfully detected with Ct values below 35.

All 6 strains were also detected in the below conditions (data is not shown).

- Mesenchymal stem cells (MSC): 0.5 x 10⁶ cells/mL
- T-cells: 1.0 x 10⁶ cells/mL

Detectable at 9 CFU/mL with cell suspension sample

<Higher sensitivity method>

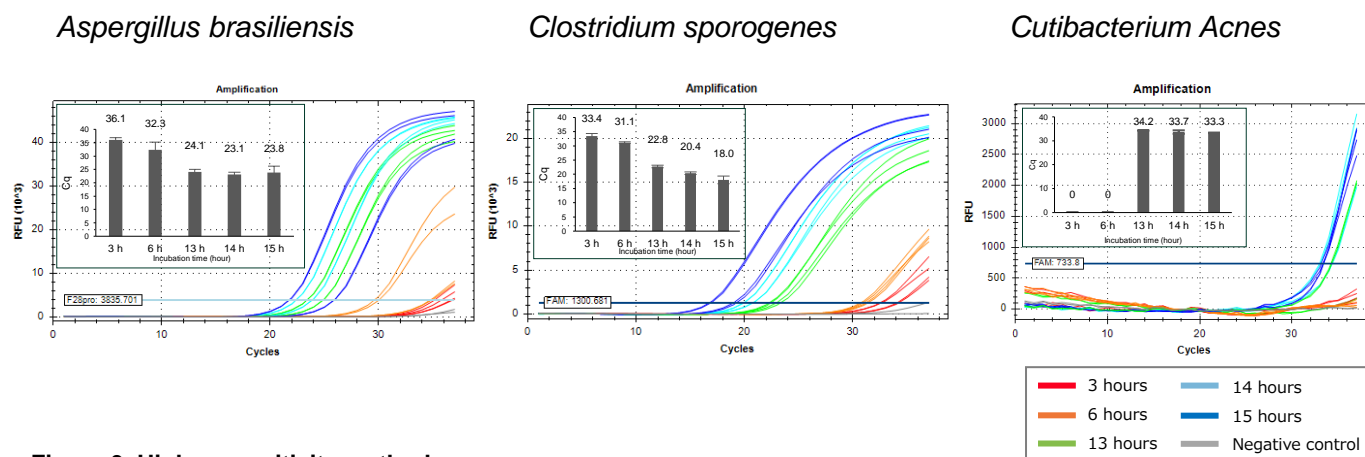


Figure 3. Higher sensitivity method

Microbial suspensions were prepared at **2 CFU** for *Aspergillus brasiliensis*, *Clostridium sporogenes* and *Cutibacterium acnes*. Although the regular protocol of RiboNAT™ specifies an incubation time of 3 hours, experiments were conducted with incubation times ranging from 3 to 15 hours. After incubation, RNA was extracted using RiboNAT™, and rRNA was detected according to the protocol. All three strains including strictly anaerobic bacteria (*Clostridium sporogenes*) and slow growth bacteria (*Cutibacterium Acnes*) were successfully detected with Ct values below 35 after 14 hours or longer incubation.

Detectable at 2 CFU/mL with 14 hours incubation

<Reduction of false positive >

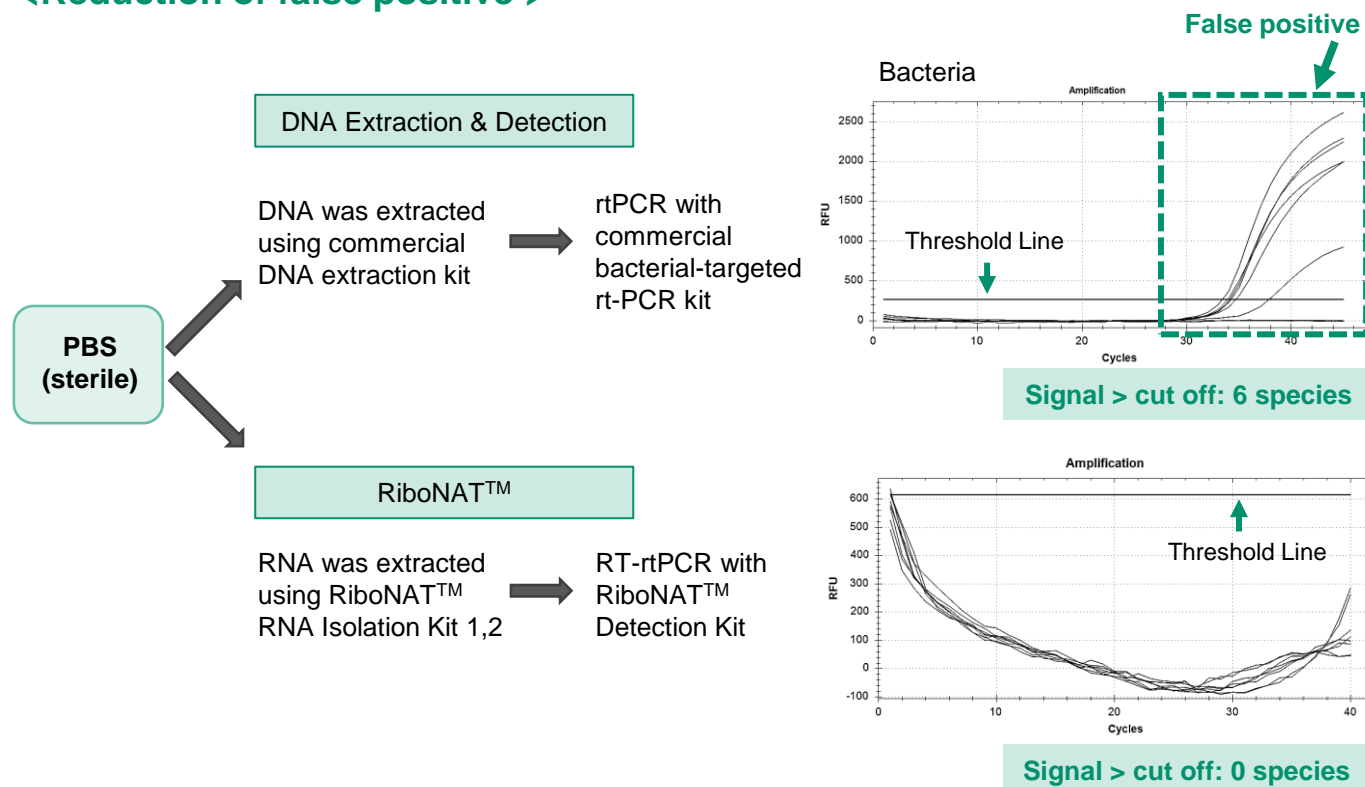


Figure 4. Reduction of false positive

Nucleic acid testing (NAT) for bacteria was performed using commercially available sterile PBS as the test sample. In one set (upper figure), DNA was extracted using a commercial kit followed by bacterial-targeted real-time PCR. In the other set (lower figure), RNA was extracted using RiboNAT™ and subjected to reverse transcription real-time PCR. While six amplification curves were observed in the DNA-based method, no amplification was detected using RiboNAT™.

RiboNAT™ can reduce false positive derived from residual DNA

<Kit Components>

■ RNA Isolation Kit 1

■ RNA Isolation Kit 2

■ Detection Kit



- ① Enzyme Enhancer
- ② Activator Solution 1 (SCDM)
- ③ Activator Solution 2 (TG) ×2
- ④ Nucleic Acid Inactivator
- ⑤ DNase Solution
- ⑥ 1st-DNase Buffer (1)
- ⑦ 2nd-DNase Buffer (2)
- ⑧ Proteinase K Solution
- ⑨ Enzyme Mix

- ① Binding Buffer
- ② 1st-Wash Buffer (1)
- ③ 2nd-Wash Buffer (2)
- ④ Lysis Buffer
- ⑤ Lytic Enhancer
- ⑥ Elution Buffer ×3
- ⑦ Magnetic Beads
- ⑧ Sample Tube ×2
- ⑨ Elution Tube

- ① Water
- ② Positive Control RNA
- ③ Internal Control RNA
- ④ Detection Mix ×2

All three kits are required for the assay.

<Protocol Video>



Please access the protocol video via the link/QR code, or search by product code number on our website (<https://labchem-wako.fujifilm.com>).

<https://labchem-wako.fujifilm.com/jp/product/detail/W01W0129-9840.html>

<Product Information>

| Product code | Product name | Size | Storage |
|--------------|---|-----------|------------------|
| 291-98401 | RiboNAT™ Rapid Sterility Test - RNA Isolation Kit 1 | 50 Tests | -20°C |
| 297-98001 | RiboNAT™ Rapid Sterility Test - RNA Isolation Kit 2 | 50 Tests | Room temperature |
| 293-98101 | RiboNAT™ Rapid Sterility Test - Detection Kit | 100 Tests | -20°C |

Listed products are intended for laboratory research use only, and not to be used for drug, food or human use.

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