

SHENTEK

Mycoplasma DNA Standards

User Guide

Version: A/0
For Research Use Only

Huzhou Shenke Biotechnology Co., Ltd.

(IMPORTANT: Please read this document carefully before experiment.)

■ Product description

The MycoSHENTEK® Mycoplasma DNA Standards consist of highly purified genomic DNA derived from mycoplasma species, which can be used as positive controls for PCR-based assays or for the construction of qPCR standard curves in qPCR applications. These standards are commonly used to determine the concentration of unknown DNA with high accuracy. As the product contains only purified genomic DNA and no viable organisms, and presents no risk of infection.

■ Kit contents and storage

Table 1. Kit components and storage

Product Name	Product No.	Reagent	Part No.	Quantity
<i>Mycoplasma orale</i> DNA Standard	1502550	<i>Mycoplasma orale</i> DNA Standard	NNA055	50 µL × 1 tube
		DNA Dilution Buffer (DDB)	NND001	1.5 mL × 1 tube
<i>Mycoplasma pneumoniae</i> DNA Standard	1502551	<i>Mycoplasma pneumoniae</i> DNA Standard	NNA054	50 µL × 1 tube
		DNA Dilution Buffer (DDB)	NND001	1.5 mL × 1 tube
<i>Mycoplasma hyorhinis</i> DNA Standard	1502552	<i>Mycoplasma hyorhinis</i> DNA Standard	NNA056	50 µL × 1 tube
		DNA Dilution Buffer (DDB)	NND001	1.5 mL × 1 tube

The kit components should be stored at -20°C for up to 24 months. Please check the expiration date on the labels.

■ Procedure

Serial dilutions of DNA standards for standard curve generation

Please check the concentration labeled on the tube of DNA Standard prior to dilution.

Dilute the DNA Standard with DDB following the serial dilution procedure below:

1. Thaw the DNA Standard and DDB completely at 2-8°C or melt on ice. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times.
2. Label six 1.5 mL microcentrifuge tubes as ST1, ST2, ST3, ST4, ST5 and ST6.
3. Prepare the ST1 tube by diluting the DNA Standard to a final concentration of 1×10^6 copies/ μ L with DDB. Calculate the required volume of DDB using the formula below, then proceed to the ST1 tube:

$$\frac{\text{DNA Control conc. (A)} \times \text{Volume of DNA Control(B)}}{1 \times 10^6 \text{ copies}/\mu\text{L}} - \text{Volume of DNA Control(B)}$$

For example:

The concentration on the label of the DNA Standard is 2.0×10^7 copies/ μ L (A), pipette 10 μ L (B) of the DNA Standard to the ST1 tube. Add the below volume to achieve a final concentration of 1×10^6 copies/ μ L.

$$\frac{2.0 \times 10^7 \text{ copies}/\mu\text{L} \times 10 \text{ uL}}{1 \times 10^6 \text{ copies}/\mu\text{L}} - 10 \text{ uL} = 190 \text{ uL}$$

4. Vortex the tube briefly, then spin for 3 - 5 seconds in a microcentrifuge to collect the solution, and repeat this step 3 times to mix thoroughly.
5. Add 90 μ L DDB to each tube of ST2, ST3, ST4, ST5 and ST6.
6. Perform the serial dilutions according to Table 2:

Table 2. DNA Standard Dilution Scheme

Serial dilution tube	Dilution	Conc. (copies/ μ L)
ST1	Dilute the DNA Standard with DDB	1×10^6
ST2	10 μ L ST1 + 90 μ L DDB	1×10^5
ST3	10 μ L ST2 + 90 μ L DDB	1×10^4
ST4	10 μ L ST3 + 90 μ L DDB	1×10^3
ST5	10 μ L ST4 + 90 μ L DDB	1×10^2
ST6	10 μ L ST5 + 90 μ L DDB	1×10^1

- The remaining unused DDB need to be stored at 2-8°C. If the solution is cloudy or contains precipitates, heat at 37°C until it clear.
- At least five concentrations of the standard curve should be included. To select

appropriate sample dilutions, we recommend performing method validation before sample testing.

■ qPCR Reaction MIX preparation

Prepare qPCR Reaction MIX for Mycoplasma DNA detection assay (Refer to product No.: 1509841) according to Table 3.

Table 3. qPCR Reaction MIX preparation

Sample	Volume/reaction
Standard curve	10 μ L qPCR MIX + 20 μ L ST1/ST2/ST3/ST4/ST5/ST6

Note:

The above is merely provided as an example only. For specific experiment preparation, qPCR program configuration and data analysis, please refer to the User Guide of the Mycoplasma DNA Detection Kit (2G) (Product No.: 1509841).

Users may choose suitable qPCR reagents and instruments at their discretion. The reagents and instruments should be properly qualified and validated for further use.

■ Attentions

1. This product is intended for research use only and is not used for clinical diagnosis or treatment. Use with appropriate caution.
2. For your safety and health, please use the product in a biosafety cabinet and wear lab clothes and disposable gloves.

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Support & Contact

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