

# Genomic & Microbial Reference Materials



- Genomic DNA extracts of defined microorganisms for specificity testing in conventional and qPCR
- PCR quantification standards of mycoplasma and bacterial genomic DNA for applications in conventional and qPCR
- 10CFU™ and 100CFU™ Sensitivity Standards for validating robustness and sensitivity of molecular methods for mycoplasma detection as required by the *European Pharmacopoeia, Chapter 2.6.7* and by the *Japanese Pharmacopoeia, Chapter G3*

# Genomic DNA Extracts



## Background & Description

- Conventional and qPCR
- Specificity testing

These preparations contain genomic DNA extracted from defined microorganisms at low passage, extracted by subsequent column absorption methods. The DNA extract is then partially sequenced to confirm identity and titrated.

## Content

1 vial with genomic DNA extract with 10 ng ± 2 ng, freeze-dried;  
1 vial with 200 µl Tris-HCl buffer (10 mM, pH 8.5) for DNA rehydration;

## Ordering Information

51-0116	<i>Acholeplasma laidlawii</i>	51-3361	Methicilin-resistant <i>Staphylococcus aureus</i> (MRSA)
2127-30007	<i>Acinetobacter bamanii</i>	2134-30164	<i>Morganella morganii</i>
2128-30187	<i>Aeromonas hydrophila</i>	51-0030	<i>Micrococcus luteus</i>
2101-00819	<i>Aspergillus fumigatus</i>	51-0129	<i>Mycoplasma arginini</i>
51-0031	<i>Bacillus cereus</i>	51-0162	<i>Mycoplasma arthritis</i>
51-0010	<i>Bacillus subtilis</i>	51-0117	<i>Mycoplasma fermentans</i>
2129-02046	<i>Bacillus thuringiensis</i>	51-0115	<i>Mycoplasma gallisepticum</i>
51-5571	<i>Bordetella pertussis</i>	51-0195	<i>Mycoplasma genitalium</i>
2130-07288	<i>Burkholderia capacia</i>	51-0111	<i>Mycoplasma hominis</i>
2102-04688	<i>Campylobacter jejuni</i>	51-0130	<i>Mycoplasma hyorhinis</i>
51-1386	<i>Candida albicans</i>	51-0112	<i>Mycoplasma orale</i>
2103-11226	<i>Candida glabrata</i>	51-1746	<i>Mycoplasma penetrans</i>
2104-11947	<i>Candida guilliermondii</i>	51-0119	<i>Mycoplasma pneumoniae</i>
2105-70624	<i>Candida haemulonii</i>	51-0124	<i>Mycoplasma synoviae</i>
2106-30039	<i>Citrobacter freundii</i>	2135-10036	<i>Neisseria meningitidis</i>
2125-90874	<i>Candida tropicalis</i>	2136-13387	<i>Proteus vulgaris</i>
2107-04595	<i>Citrobacter koseri</i>	51-0071	<i>Pseudomonas aeruginosa</i>
51-0792	<i>Clostridium acetobutylicum</i>	2116-04479	<i>Proteus mirabilis</i>
2108-00756	<i>Clostridium perfringens</i>	51-7058	<i>Salmonella enterica</i>
51-0053	<i>Enterobacter aerogenes</i>	2117-30121	<i>Serratia marcescens</i>
2110-30054	<i>Enterobacter cloacae</i>	2137-04782	<i>Shigella flexneri</i>
2111-20680	<i>Enterococcus casseliflavus</i>	2138-05570	<i>Shigella sonnei</i>
2112-30633	<i>Enterococcus durans</i>	51-0164	<i>Spiroplasma citri</i>
51-0478	<i>Enterococcus faecalis</i>	51-0231	<i>Staphylococcus aureus</i>
2113-20477	<i>Enterococcus faecium</i>	51-0044	<i>Staphylococcus epidermidis</i>
2114-20160	<i>Enterococcus hirse</i>	2118-20328	<i>Staphylococcus hominis</i>
51-0083	<i>Escherichia coli</i>	2119-20263	<i>Staphylococcus haemolyticus</i>
2115-08579	<i>Escherichia coli</i> O157:H7	2122-20480	<i>Streptococcus bovis</i>
51-1368	<i>Fluoribacter bozemaniae</i> (syn. <i>Legionella bozemaniae</i> )	2123-06176	<i>Streptococcus dysgalactiae</i>
2131-05934	<i>Geobacillus stearothermophilus</i>	2126-20523	<i>Streptococcus mutans</i>
2132-30104	<i>Klebsiella pneumoniae</i>	51-0566	<i>Streptococcus pneumoniae</i>
2133-70603	<i>Klebsiella pneumoniae</i> , ESBL+	2139-20068	<i>Streptococcus sanguinis</i>
51-1723	<i>Lactobacillus acidophilus</i>	2120-20229	<i>Staphylococcus saprophyticus</i>
51-1370	<i>Legionella dumofii</i>	2121-50170	<i>Stenotrophomonas maltophilia</i>
51-1533	<i>Legionella jordanis</i>	51-0177	<i>Ureaplasma urealyticum</i>
51-0101	<i>Legionella pneumophila</i>	2140-04780	<i>Yersinia enterocolitica</i>
51-1514	<i>Legionella pneumophila subsp. fraseri</i>	2141-08992	<i>Yersinia pseudotuberculosis</i>
51-1515	<i>Legionella pneumophila subsp. pascullei</i>		

# PCR Quantification Standards



## Background & Description

- Conventional and qPCR
- Standard curves, evaluation of assay performance
- Preparation of dilution series for quantification
- Low titer (e.g.  $3 \times \text{LOD}_{95}$ ) controls

Standardization and quantification of nucleic acid detection is a difficult task as there are no reliable standards for low nucleic acid copy numbers. Detection methods such as PCR and NASBA are prone to inhibition caused by many substances commonly found in plant and animal tissues, food matrices and extraction solutions. When co-purified with DNA or RNA, inhibitors reduce amplification efficiency, causing an underestimation of the quantity of target nucleic acid or even false-negative results.

Minerva Biolabs calibration reagents contain genomic DNA extract from low passage and defined microorganisms. The DNA is extracted by subsequent column absorption methods. The DNA extract was partially sequenced and the sequence aligned to confirm identity. Titration was done after photometric quantification of the preparation standard and dsDNA fluorometric quantification against a synthetic standard.

By using the PCR Quantification Standards, qPCR users can include a precise low count of DNA copies in their assays. This will serve as an exact reference value for the estimation of detection limits and for the comparison between different detection methods.

## Content

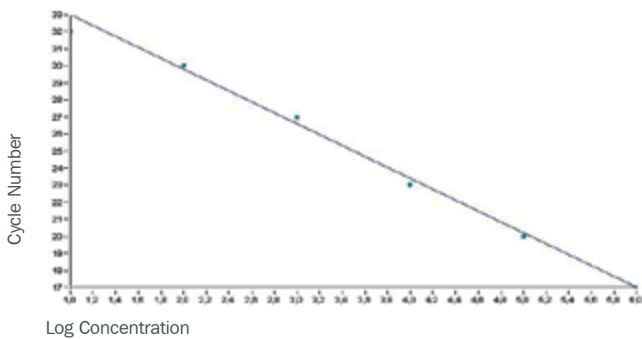
1 vial with DNA,  $1 \times 10^8$  genome copies, freeze-dried;  
3 vials with 2 ml of Tris-HCl buffer (10 mM, pH 8.5) for rehydration and preparation of serial dilutions;

## Ordering Information

52-0116	<i>Acholeplasma laidlawii</i>
52-5571	<i>Bordetella pertussis</i>
52-0083	<i>Escherichia coli</i>
52-0101	<i>Legionella pneumophila</i>
52-0129	<i>Mycoplasma arginini</i>
52-0117	<i>Mycoplasma fermentans</i>
52-0115	<i>Mycoplasma gallisepticum</i>

52-0130	<i>Mycoplasma hyorhinis</i>
52-0112	<i>Mycoplasma orale</i>
52-0119	<i>Mycoplasma pneumoniae</i>
52-0124	<i>Mycoplasma synoviae</i>
52-0164	<i>Spiroplasma citri</i>
52-0071	<i>Pseudomonas aeruginosa</i>
52-0103	<i>Mycoplasma salivarium</i>

Standard Curve



Real-time Amplification Plot

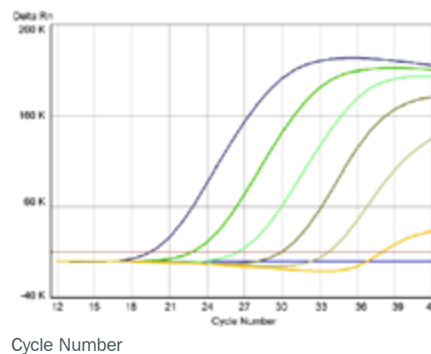


Fig. Quantification of *Mycoplasma pneumoniae* DNA. Logarithmic plot of fluorescence vs cycle number (Venor<sup>®</sup>GeM qEP, platform: ABI Prism<sup>®</sup> 7500). Template DNA ranging from  $2 \times 10^5$  - 2 genome equivalents.

# 10CFU™ Sensitivity Standards



For validating robustness and detection limit of molecular mycoplasma test methods in presence of the sample matrix.

## Background & Description

The European Pharmacopoeia (EP 2.6.7), the Japanese Pharmacopoeia (JP G3) and the US Pharmacopoeia (USP <71>) define the required sensitivity for nucleic acid-based assays (e.g. PCR) as valid detection methods for microbiological testing of biopharmaceuticals. According to these guidelines, a test sensitivity of 10 colony-forming units (CFU) per ml of sample volume (or min. 100 CFU/ml for USP) must be reached by the performing lab as part of the robustness testing, in presence of the sample matrix.

Each vial of 10CFU™ Sensitivity Standards contains 10 CFU of inactivated mycoplasma, which can be safely and reliably used in cell culture and production facilities, where a microbiology lab is not available. Once resuspended in the sample matrix of interest, these samples must be tested positive by the applied method. Please note that due to the mycoplasma inactivation, the 10CFU™ Sensitivity Standards are not suitable for the culture method. Extensive proficiency tests indicated that mycoplasma DNA extraction is indispensable to achieve highest sensitivity by PCR-based methods. After extraction, the 10CFU™ extract can directly be used for PCR. The genome units (GU) to CFU ratio is provided in The Certificate of Analysis for each lot.

## Content

Unit package: 3 vials with 10 CFU of the corresponding mycoplasma species ; 2 vials with negative controls;  
Set package (Mycoplasma set Cat. No. 102-0002):  
2 vials with 10 CFU of each mycoplasma species listed in the EP 2.6.7 (18 vials in total; *M. salivarium* is not included in the set). 2 vials with negative controls;

## Ordering Information

Cat. No. 102-8003 *Acholeplasma laidlawii*

Cat. No. 102-1003 *Mycoplasma arginini*

Cat. No. 102-6003 *Mycoplasma fermentans*

Cat. No. 102-3003 *Mycoplasma gallisepticum*

Cat. No. 102-7003 *Mycoplasma hyorhinis*

Cat. No. 102-2003 *Mycoplasma orale*

Cat. No. 102-4003 *Mycoplasma pneumoniae*

Cat. No. 102-5003 *Mycoplasma synoviae*

Cat. No. 102-1103 *Mycoplasma salivarium*

Cat. No. 102-9003 *Spiroplasma citri*

Cat. No. 102-0002 Mycoplasma Set

# 100CFU™ Sensitivity Standards

Additional products for validating robustness and detection limit of molecular mycoplasma test methods in presence of a specific sample matrix.

## Background & Description

100CFU™ Sensitivity Standards are designed to support the validation of nucleic acid amplification technology (NAT)-based tests for mycoplasma detection, according to USP <71>.

More generally, including this supplementary concentration in the matrix validation procedure significantly improves the confidence of the test method.

- Irreversibly inactivated mycoplasma in an amount corresponding to 100 CFU
- Additional test concentration to the 10CFU™ Sensitivity Standards for the validation of PCR-based detection tests.
- The genome units (GU) to CFU ratio is provided in The Certificate of Analysis for each lot.

## Content

Unit package: 3 vials with 100 CFU of the corresponding mycoplasma species; 2 vials with negative controls;

## Ordering Information

Cat. No. 103-8003 *Acholeplasma laidlawii*

Cat. No. 103-1003 *Mycoplasma arginini*

Cat. No. 103-6003 *Mycoplasma fermentans*

Cat. No. 103-3003 *Mycoplasma gallisepticum*

Cat. No. 103-7003 *Mycoplasma hyorhinis*

Cat. No. 103-2003 *Mycoplasma orale*

Cat. No. 103-4003 *Mycoplasma pneumoniae*

Cat. No. 103-1103 *Mycoplasma salivarium*

Cat. No. 103-5003 *Mycoplasma synoviae*

Cat. No. 103-9003 *Spiroplasma citri*

## How to order

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