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™ 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*

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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	*Acholeplasma laidlawii*
2127-30007	*Acinetobacter bamanii*
2128-30187	*Aeromonas hydrophila*
2101-00819	*Aspergillus fumigatus*
51-0031	*Bacillus cereus*
51-0010	*Bacillus subtilis*
2129-02046	*Bacillus thueringiensis*
51-5571	*Bordetella pertussis*
2130-07288	*Burkholderia capacia*
2102-04688	*Campylobacter jejuni*
51-1386	*Candida albicans*
2103-11226	*Candida glabrata*
2104-11947	*Candida guiliermondii*
2105-70624	*Candida haemulonii*
2125-90874	*Candida tropicalis*
2106-30039	*Citrobacter freundii*
2107-04595	*Citrobacter koseri*
51-0792	*Clostridium acetobutylicum*
2108-00756	*Clostridium perfringens*
51-0053	*Enterobacter aerogenes*
2110-30054	*Enterobacter cloacae*
2111-20680	*Enterococcus casseliflavus*
2112-30633	*Enterococcus durans*
51-0478	*Enterococcus faecalis*
2113-20477	*Enterococcus faecium*
2114-20160	*Enterococcus hirse*
51-0083	*Escherichia coli*
2115-08579	*Escherichia coli 0157:H7*
51-1368	*Fluoribacter bozemanae (syn. Legionella bozemanae)*
2131-05934	*Geobacillus stearothermophilus*
2132-30104	*Klebsiella pneumoniae*
2133-70603	*Klebsiella pneumoniae, ESBL+*
51-1723	*Lactobacillus acidophilus*
51-1370	*Legionella dumofii*
51-1533	*Legionella jordanis*
51-0101	*Legionella pneumophila*
51-1514	*Legionella pneumophila subsp. fraseri*
51-1515	*Legionella pneumophila subsp. pascullei*
51-3361	*Methicilin-resistant Staphylococcus aureus (MRSA)*
2134-30164	*Morganella morganii*
51-0030	*Micrococcus luteus*
51-0129	*Mycoplasma arginini*
51-0162	*Mycoplasma arthritidis*
51-0117	*Mycoplasma fermentans*
51-0115	*Mycoplasma gallisepticum*
51-0195	*Mycoplasma genitalium*
51-0111	*Mycoplasma hominis*
51-0130	*Mycoplasma hyorhinis*
51-0112	*Mycoplasma orale*
51-1746	*Mycoplasma penetrans*
51-0119	*Mycoplasma pneumoniae*
51-0124	*Mycoplasma synoviae*
2135-10036	*Neisseria meningitidis*
2116-04479	*Proteus mirabilis*
2136-13387	*Proteus vulgaris*
51-0071	*Pseudomonas aeruginosa*
51-7058	*Salmonella enterica*
2117-30121	*Serratia marcescens*
2137-04782	*Shigella flexneri*
2138-05570	*Shigella sonnei*
51-0164	*Spiroplasma citri*
51-0231	*Staphylococcus aureus*
51-0044	*Staphylococcus epidermidis*
2118-20328	*Staphylococcus hominis*
2119-20263	*Staphylococcus haemolyticus*
2120-20229	*Staphylococcus saprophyticus*
2121-50170	*Stenotrophomonas maltophilia*
2122-20480	*Streptococcus bovis*
2123-06176	*Streptococcus dysgalactiae*
2126-20523	*Streptococcus mutans*
51-0566	*Streptococcus pneumoniae*
2139-20068	*Streptococcus sanguinis*
51-0177	*Ureaplasma urealyticum*
2140-04780	*Yersinia enterocolitica*
2141-08992	*Yersinia pseudotuberculosis*

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 x LOD_{95}) controls

Standardization of quantitative nucleic acid detection is complicated by the lack of reliable standards for nucleic acids´ low copy numbers. Substances commonly found in sample matrices like plant and animal tissues, food matrices and extraction solutions often cause inhibition of detection reactions such as PCR and NASBA. 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 extracted by column absorption methods from defined microorganisms at low passage. The identity of the species is confirmed by partial sequencing and titration is performed 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.

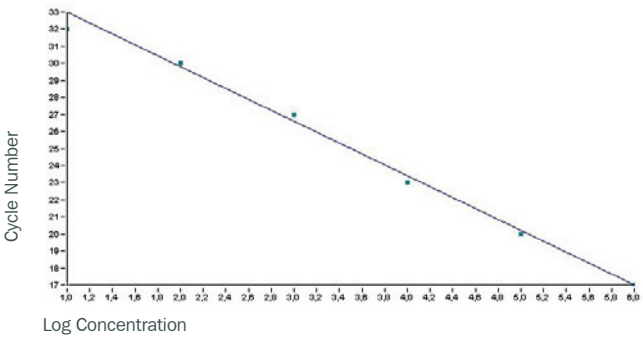
Content

1 vial with DNA, $1x10^8$ 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;

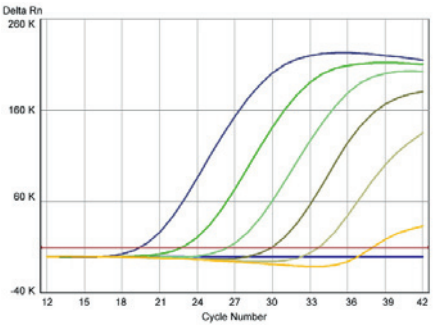
Ordering Information

52-0116	*Acholeplasma laidlawii*
52-5571	*Bordetella pertussis*
52-0083	*Escherichia coli*
52-0101	*Legionella pneumophila*
52-0129	*Mycoplasma arginini*
52-0117	*Mycoplasma fermentans*
52-0115	*Mycoplasma gallisepticum*
52-0130	*Mycoplasma hyorhinis*
52-0112	*Mycoplasma orale*
52-0119	*Mycoplasma pneumoniae*
52-0103	*Mycoplasma salivarium*
52-0124	*Mycoplasma synoviae*
52-0071	*Pseudomonas aeruginosa*
52-0164	*Spiroplasma citri*

Standard Curve



Real-time Amplification Plot



Cycle Number

Fig. Quantification of *Mycoplasma pneumoniae* DNA. Logarithmic plot of fluorescence vs cycle number (Venor®GeM qEP, platform: ABI Prism® 7500). Template DNA ranging from $2x10^5$ - 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 Chapter 2.6.7 (EP 2.6.7) and the Japanese Pharmacopoeia (17th Edition) Chapter G3 (JP G3) describe NAT-based mycoplasma tests such as PCR. Both require to show the detection of 10 Colony Forming Units per ml of sample volume (CFU/ml), if said tests are proposed as alternative methods to traditional culture assays. Such sensitivity must be demonstrated as part of the robustness testing for each specific sample matrix of interest. However, since most cell culture labs and production facilities do not have access to a microbiology lab, culturing or handling viable mycoplasma colonies to use as reference tools for sensitivity testing is inadmissible.

Our 10CFU™ Sensitivity Standards contain irreversibly inactivated mycoplasma in an amount corresponding to 10 CFU and allow safe and reliable validation.

All strains are cultivated in low passages.

Grown in culture medium as described in EP 2.6.7, the 10CFU™ Sensitivity Standards are then titrated and plated for CFU determination. In order to ensure a high ratio of viable to non-viable mycoplasma and therefore a low ratio of GU* to CFU, the mycoplasma are harvested in the logarithmic growth phase.

Once resuspended in the sample matrix of interest (as in EP 2.6.7/ JP G3), each vial of 10CFU™ Sensitivity Standards must be positively tested 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 testing indicated that mycoplasma DNA extraction is indispensable to achieve highest sensitivity by PCR-based methods. After extraction the 10CFU™ extract can be directly used for PCR.

* Please note: This standard material is not titrated for genome copies (GU) as EP 2.6.7/ JP G3 do not provide sensitivity limits on DNA level. No guarantee for a particular GU:CFU ratio is provided with this product and the ratio may vary from lot to 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. arginini, M. orale, M. gallisepticum, M. pneumoniae, M. synoviae, M. fermentas, M. hyorhinis, A. laidlawii, S. citri*). *M. salivarium* is not included in the set;
2 vials with negative controls;

Ordering Information

102-1003	*Mycoplasma arginini*
102-2003	*Mycoplasma orale*
102-3003	*Mycoplasma gallisepticum*
102-4003	*Mycoplasma pneumoniae*
102-5003	*Mycoplasma synoviae*
102-6003	*Mycoplasma fermentans*
102-7003	*Mycoplasma hyorhinis*
102-8003	*Acholeplasma laidlawii*
102-9003	*Spiroplasma citri*
102-1103	*Mycoplasma salivarium*
102-0002	Mycoplasma Set

How to order

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