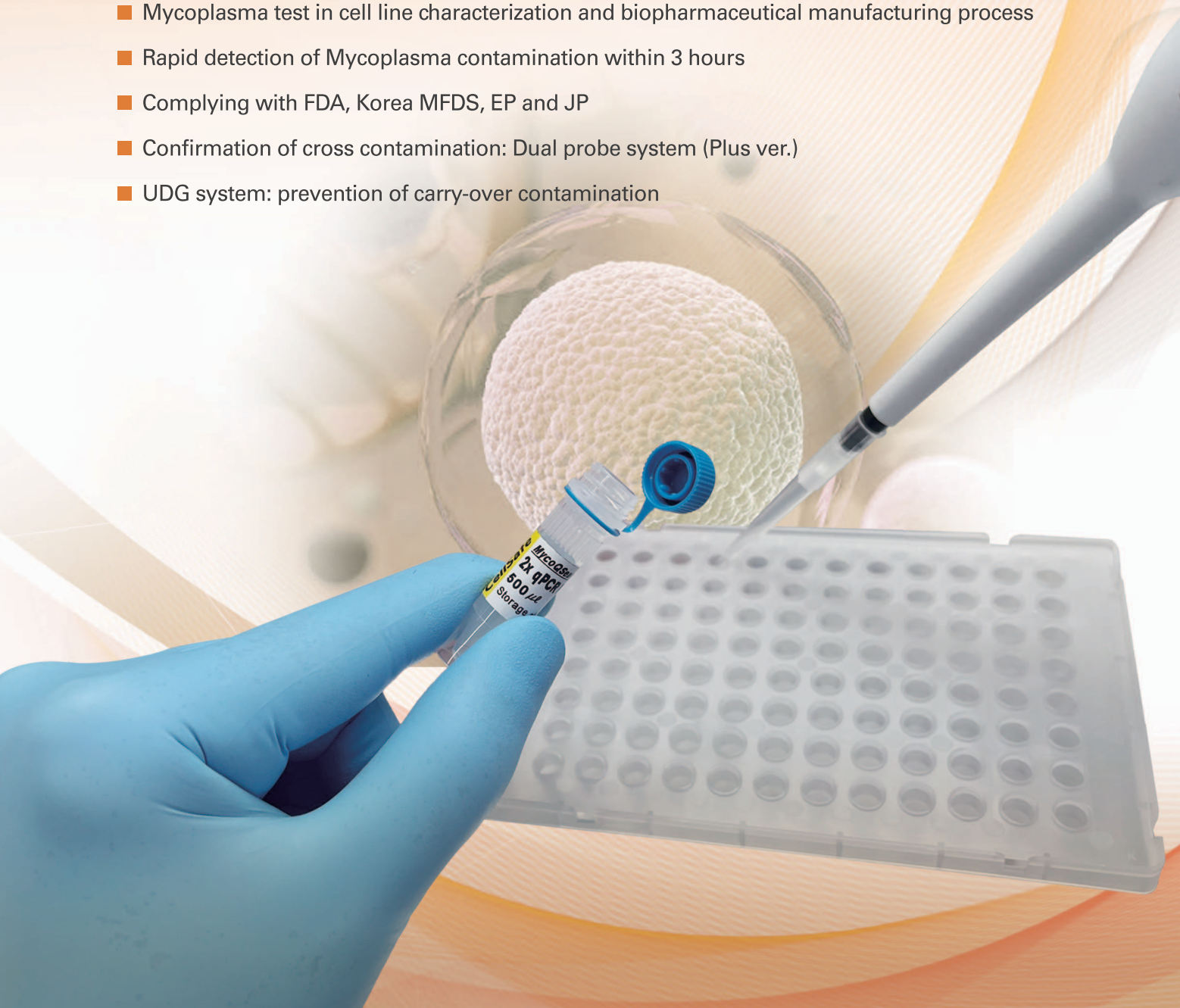
CellSafe

Validation of NAT method for the detection of Mycoplasma

- Mycoplasma test in cell line characterization and biopharmaceutical manufacturing process
- Rapid detection of Mycoplasma contamination within 3 hours
- Complying with FDA, Korea MFDS, EP and JP
- Confirmation of cross contamination: Dual probe system (Plus ver.)
- UDG system: prevention of carry-over contamination



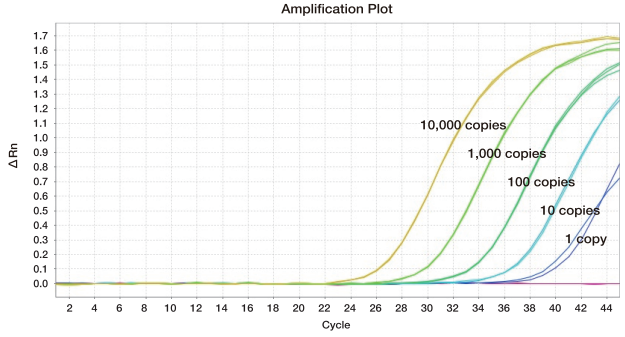
Background

Mycoplasma contamination poses a significant challenge in the production of biopharmaceuticals, including cell therapies and vaccines, as it can compromise product quantity and quality. Some strains of Mycoplasma are even pathogenic, causing respiratory and urinary tract infections. Traditionally, direct culture method and the indicator cell culture method have been used for mycoplasma tests. However, due to long cultivation time (28 days) and the difficulties of culturing different strains of mycoplasma, these methods have their own limitations to apply in the production of cell and gene therapies. Nucleic acid amplification technique (NAT) is being used as an alternative to conventional culture methods especially for the qualification of biopharmaceuticals such as ATMPs (Advanced Therapy Medicinal Products) after suitable validations are performed. To replace conventional culture methods according to guidelines like the European Pharmacopoeia (EP 2.6.7) and the Korean Ministry of Food and Drug Safety (MFDS), a high level of sensitivity and specificity, capable of detecting Mycoplasma down to 10 CFU/mL, is required.

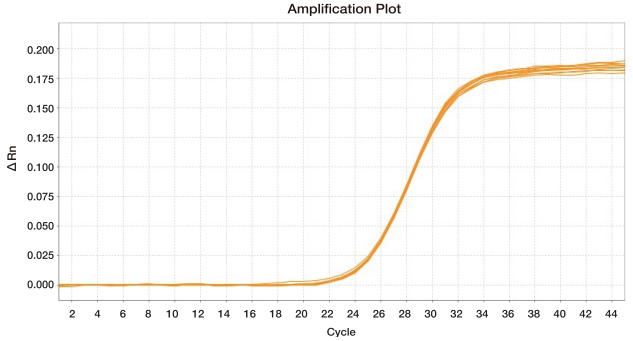
Complying with FDA, EU, Korea MFDS Guidelines based on high sensitivity and specificity

The *MycoQSearch™* Mycoplasma qPCR Detection Kit is used to detect Mycoplasma contamination of cell cultures by real-time quantitative PCR (qPCR) using hydrolysis probe. The Primer and Probe Mix included in the kit contains FAM labeled probe specific for mycoplasma species and HEX labeled probe for internal control DNA. The primer set is specific to the highly conserved 16S rRNA coding region in the mycoplasma genome. This allows for detection of *M. orale, M. hyorhinis, M. arginini, M. fermentans, Acholeplasma laidlawii*, and *M. hominis* which are primary contaminants in cell cultures as well as most other mycoplasma species including *M. pneumoniae, M. salivarium, M. synoviae, Spiroplasma. citri,* and *Ureaplasma*. However, bacterial DNA from prokaryotic cells such as *E. coli* is not amplified. The Kit can detect Mycoplasma contamination in cell cultures within 3 hours and meets the sensitivity requirements (10 CFU/mL) set by current guidelines.

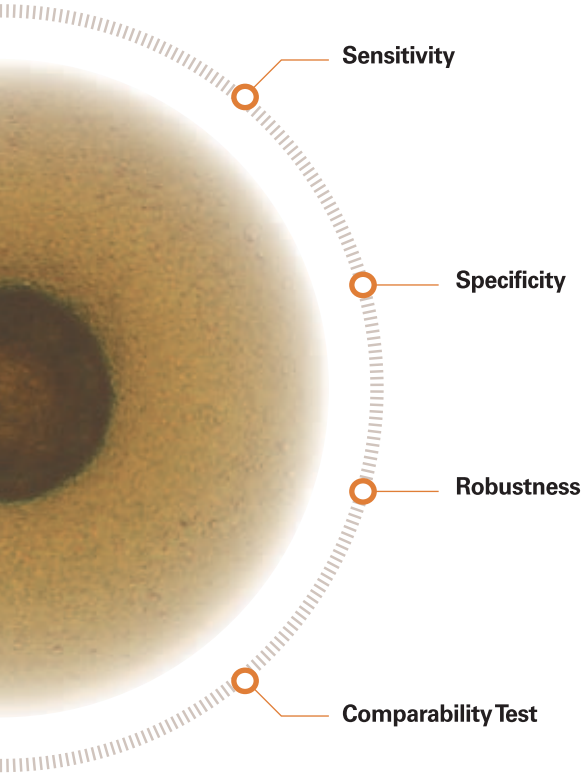
Product name	Cat. No
MycoQSearch™ Mycoplasma qPCR Detection Kit (EP)	QDEP-100
MycoQSearch™ Plus Mycoplasma qPCR Detection Kit (EP)	QDPEP-100



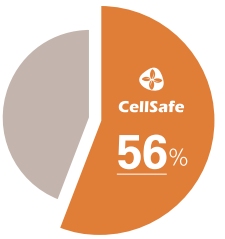
FAM Channel, Mycoplasma detection



HEX Channel, Internal Amplification Control (IAC)



A Reliable Choice for ATMP Companies Worldwide



CellSafe's Domestic Market Share

Remarkable 56% of Korean Advanced Therapy Medicinal Product (ATMP) companies have already embraced the reliability and effectiveness of CellSafe's *MycoQSearch™* Mycoplasma qPCR Detection Kit. In fact, numerous esteemed customers, including the largest cell therapy company in Korea, have chosen *MycoQSearch™* as their go-to solution to meet their stringent regulatory requirements for biomedicines. Their successful validation experiences are a testament to the exceptional performance and reliability of our kit.

Production of Mycoplasma Reference Standards for Validation of NAT Method

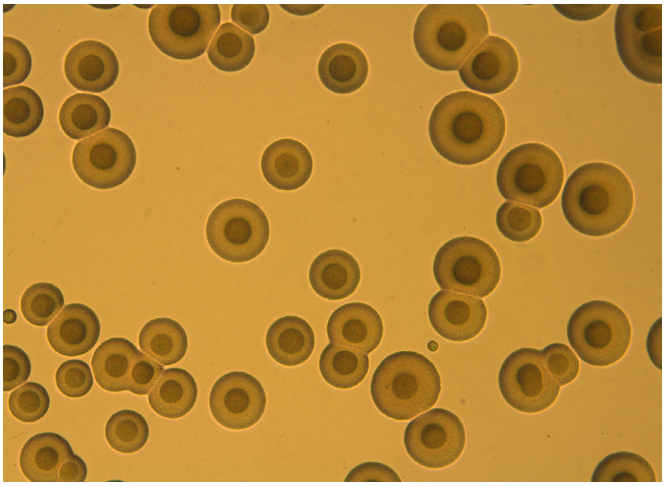
CellSafe provides mycoplasma reference standards either in CFUs or nucleic acid copies. Mycoplasma 10CFU&100CFU Standard contains inactivated mycoplasma for safe usage without environmental contamination concerns. These standards are used for comparative tests with culture method as well as for the validation of robustness and the sensitivity for NAT-based mycoplasma tests. Furthermore, Quantitative Mycoplasma Genomic DNA (100,000 copies) which is accurately quantified using digital PCR is very useful in the verification of detection limits. The Certificate of Analysis provides information on the genome copies (GC) / Colony Forming Unit (CFU) ratio for each lot.

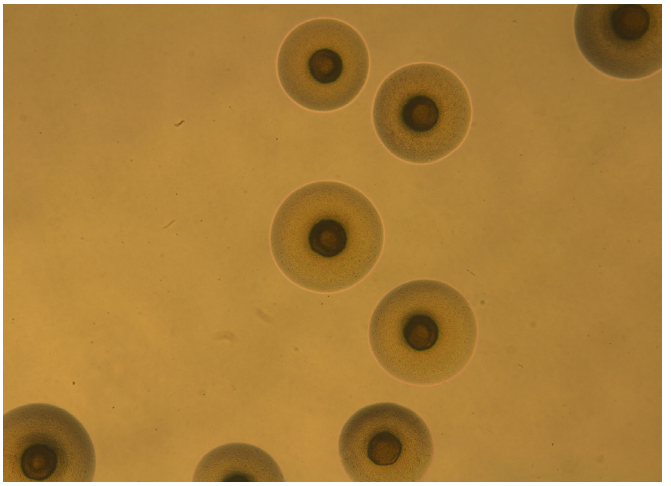
CFU

Product name	Cat. No
Mycoplasma 10 CFU *M. arginini*	M10CFU-MA
Mycoplasma 10 CFU *A. laidlawii*	M10CFU-AL
Mycoplasma 10 CFU *M. fermentans*	M10CFU-MF
Mycoplasma 10 CFU *M. hyorhinis*	M10CFU-MH
Mycoplasma 10 CFU *M. orale*	M10CFU-MO
Mycoplasma 10 CFU *M. pneumoniae*	M10CFU-MP
Mycoplasma 100 CFU *M. arginini*	M100CFU-MA
Mycoplasma 100 CFU *A. laidlawii*	M100CFU-AL
Mycoplasma 100 CFU *M. fermentans*	M100CFU-MF
Mycoplasma 100 CFU *M. hyorhinis*	M100CFU-MH
Mycoplasma 100 CFU *M. orale*	M100CFU-MO
Mycoplasma 100 CFU *M. pneumoniae*	M100CFU-MP

gDNA

Product name	Cat. No
Quantitative *M. arginini* Genomic DNA	QAGD
Quantitative *A. laidlawii* Genomic DNA	QLGD
Quantitative *M. fermentans* Genomic DNA	QFGD
Quantitative *M. hyorhinis* Genomic DNA	QHGD
Quantitative *M. orale* Genomic DNA	QOGD
Quantitative *M. pneumoniae* Genomic DNA	QPGD





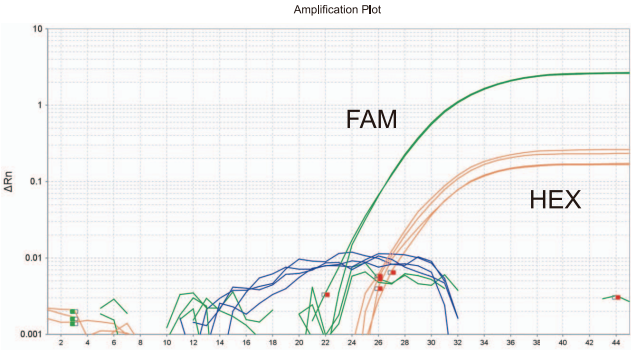
Prevent False Negatives through Internal Controls in DNA Extraction

The Internal Amplification Control (IAC) is included in the Kit to ensure the consistency of PCR reaction and monitor the presence of PCR inhibition. Acting as an overall control for DNA extraction and amplification, the IAC can be added to the test material before the DNA extraction process and PCR analysis to verify the entire procedure. This verification serves to prevent false negative results by ensuring that any mycoplasma present in the sample is not lost during the DNA extraction process.

Top-notch prevention system of mycoplasma contamination

Dual Probe System: Prevent cross contamination

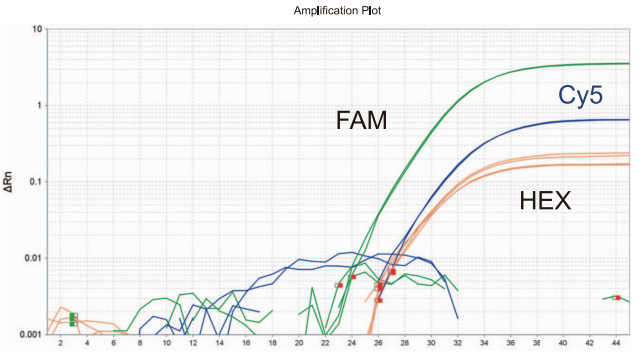
False positive results can occur due to the inflow of positive control DNA into the test samples which is caused by lab worker's mistakes or aerosols. CellSafe's Mycoplasma Detection Kit uses Cy5-labeled probe for positive control DNA, FAM (for mycoplasma species) and HEX (for internal control DNA). It is easy and convenient to confirm the cross contamination induced by the inflow of positive control DNA.



Mycoplasma contamination

UDG System: Prevent carry-over contamination

False positive results are caused by the inflow of PCR amplicon generated from the previous PCR reactions into the test samples. CellSafe's Mycoplasma Detection Kit contains dUTP instead of dTTP. The risk of amplicon contamination is reduced by using UDG (Uracil-DNA-glycosylase, not included in the kit) prior to PCR reaction. UDG removes uracil from DNA molecule by hydrolyzing the N-glycosidic bond of dUTP which results in its inactivation during the initial denaturation step. Hydrolyzed DNA can no longer function as a PCR template. Natural DNA does not contain uracil so that it is not degraded by UDG.



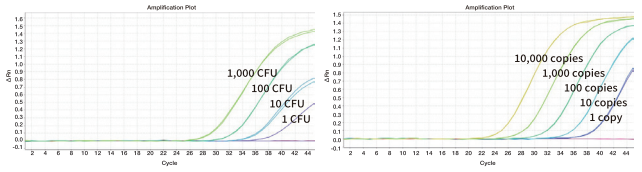
Positive control DNA contamination

Sensitivity

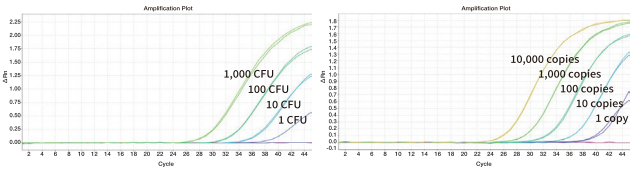
The Nucleic acid amplification technique (NAT) system is required to detect 10 CFU/mL for each mycoplasma strain to replace conventional culture methods. However, PCR technology only detects genome copies (GC), and genomic copies per colony-forming units (CFU) varies for each species and under different culture conditions. While various commercial kits for Mycoplasma detection kits may appear to have the same detection limit of 10 CFU/mL, in reality, the sensitivity of Real-Time PCR actually varies depending on the genomic copy/CFU ratio.

The Sensitivity of the PCR using CellSafe's *MycoQSearch™* Mycoplasma qPCR Detection kit is 1-10 copies/reaction (1-10 CFU/mL). We use both mycoplasma reference strain and genomic DNA reference standards to determine the sensitivity and ensure the quality of the kit.

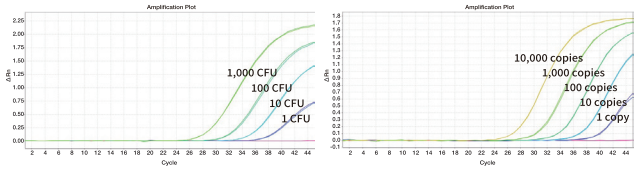
A. laidlawii



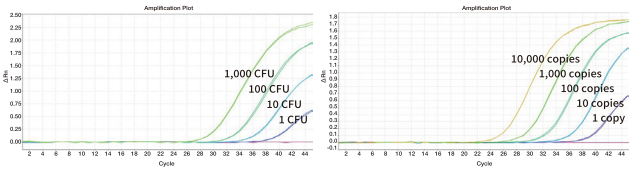
M. arginini



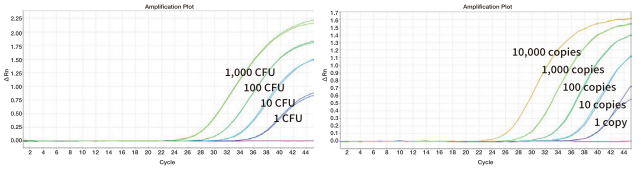
M. fermentans



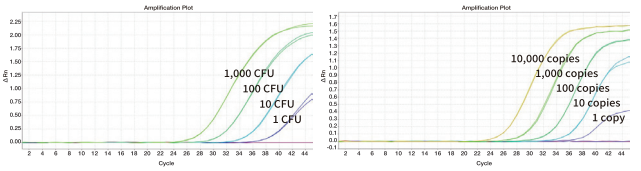
M. hyorhinis



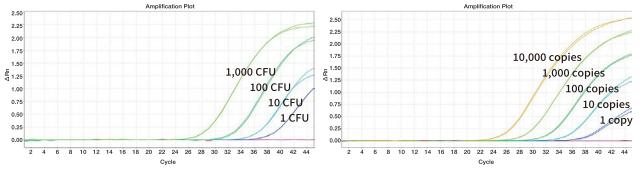
M. orale



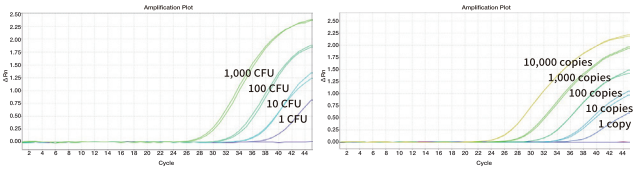
M. pneumoniae



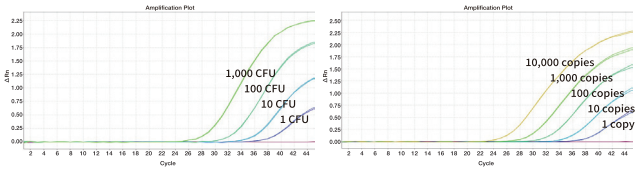
M. gallisepticum



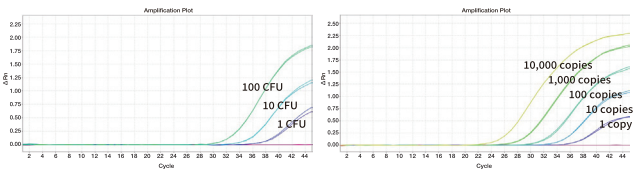
M. salivarium



M. synoviae



S. citri



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