

APPLICATION NOTE

UNIVERSAL MYCOPLASMA DETECTION KIT ATCC® 30-1012K™

Check your cultures for mycoplasma contamination with the PCR-based Universal Mycoplasma Detection Kit from ATCC

Mycoplasma contamination affects roughly 15-35% of continuous cell cultures.^{1,2} The deleterious effects of these types of contamination include the induction of chromosomal abnormalities, the disruption of DNA and RNA synthesis, and the inhibition of both cell metabolism and growth rate. Therefore, mycoplasma contamination constitutes a serious concern for cell culturists.^{1,3,4}

Mycoplasmas are classified as Mollicutes, which are a distinct class of bacteria distinguishable by their small size (ranging from 0.2 to 0.8 µm) and complete lack of a cell wall. Over 190 species of mycoplasma have been identified, but of these, just eight are responsible for approximately 95% of all cell culture contaminating events.^{4,5} Commonly, contamination occurs through a cross contaminating event, such as occurs when laboratory personnel handle both clean and contaminated media or cell cultures at the same time.¹ Importantly, these contaminating events may be difficult to detect or prevent. For example, mycoplasma can grow to densities of 10⁷-10⁸ organisms/mL without affecting obvious changes in the turbidity or pH of the culture media.⁶ Further, they have a small genome (580 kb to 2,220 kb) and correspondingly few metabolic pathways that are active enough to reveal their presence.⁶ Furthermore, because mycoplasma do not possess a cell wall, the majority of commonly used antibiotics are not effective at preventing their growth should a contaminating event occur. Therefore, the best protection against mycoplasma is to identify a contaminated culture quickly, before the contamination spreads to other cultures. To facilitate the rapid and reliable detection of mycoplasma in cell culture, ATCC offers the PCR-based Universal Mycoplasma Detection Kit (ATCC [30-1012K](#)).

The Universal Mycoplasma Detection Kit is able to detect a wide range of Mollicutes infections, including the eight species most likely to contaminate cell cultures (*M. arginini*, *M. fermentans*, *M. hominis*, *M. hyorhinis*, *M. orale*, *M. pirum*, *M. salivarium*, and *A. laidlawii*), as well as species from other Mollicutes genera like *Acholeplasma*, *Spiroplasma*, and *Ureaplasma*. To achieve detection over a wide range of species, the kit uses universal primers specific to the conserved 16S rRNA coding region of the mycoplasma genome, a thermo-stable *Taq*-polymerase, and a touchdown PCR approach.⁷ The touchdown PCR protocol employs a high annealing temperature in the initial cycle that decreases with subsequent cycles. This protocol increases the likelihood of primers binding to the specific targets and reducing the likelihood that non-specific targets (ie, from cells or other bacteria like *E. coli*) will be amplified. Using this method, mycoplasma contamination is easily



Universal Mycoplasma Detection Kit (ATCC No. [30-1012K](#)).

recognized as a distinct PCR product ranging in size from 434 to 468 bp on an agarose gel, and the assay is sensitive to as few as 20 genome copies.

The Universal Mycoplasma Detection Kit comes complete with a proprietary mix of primers, buffers, dNTPs and thermo-stable polymerase that have been optimized to generate the consistent, reliable results, in a convenient and easy to use format. The kit has everything you need to keep mycoplasma contamination from spreading through your cell cultures, and affecting your research. Visit www.atcc.com or call ATCC to get more information and to order your kit today.

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