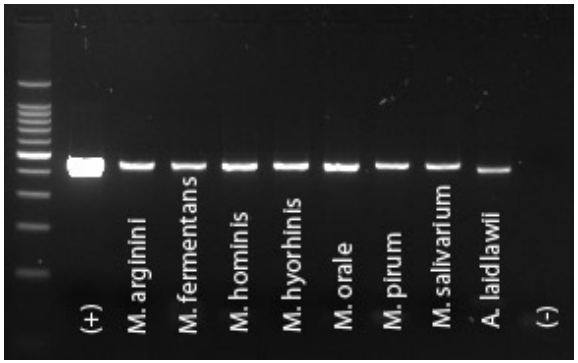
GEL ELECTROPHORESIS PROTOCOL

Step	
1	Prepare a 3% agarose gel.
2	Prepare samples: Add 10 µL of the PCR product to 1.5 µL loading buffer. Mix thoroughly.
3	Load samples and a DNA marker (e.g., 100 bp ladder) onto the gel.
3	Electrophorese until the tracking dye migrates 60-70% the length of the gel.
4	Stain the gel with ethidium bromide or similar stain and view with UV illumination.

Results: A test sample that is positive for the presence of mycoplasma shows a distinct band at 434 to 468 bp. The positive control samples exhibit a 464-bp band. There should be no visible band in the negative control lane.

DETECTION OF TOP 8 MYCOPLASMA SPECIES



Detection of the top eight mycoplasma species that infect cell cultures is shown. Distinct bands in the 434 bp to 468 bp range confirm the presence of mycoplasma.

The first lane is a 100 bp DNA ladder with a highlighted band at 500 bp. The second lane is 2.5 pg of the positive control (*M. arginini* chromosomal DNA) displaying a 464-bp PCR product. Cultures contaminated with mycoplasma typically generate signals similar to the positive control and at least as strong as those shown here.

TROUBLESHOOTING*

Problem	Potential Cause	Solution
Positive control does not exhibit a 464-bp band.	PCR did not work.	Check to make sure the touchdown protocol was programmed correctly in the thermal cycler.
Negative control lane shows a 464-bp band.	Contamination during preparation of the PCR samples.	Prepare new samples and repeat PCR. If possible, use a dedicated PCR work station with laminar flow or a laminar flow hood to avoid environmental contamination.
No bands present in the sample or positive control + lysate lanes, but a 464-bp band observed in the positive control lane.	Inhibition of PCR by the cell lysate, which suggests that too many cells were used in the assay.	If more than 10^5 cells were used, thaw cell extract and dilute to 10^5 cells per 50 µL with Lysis Buffer. If the number of cells is unknown, then dilute the extract 1 to 5 and 1 to 10 with Lysis Buffer. Repeat the lysis step in the Sample Preparation Protocol. Perform PCR with 2.5 µL of the diluted extracts.
Bands outside the 434 to 468 bp range are observed in the PCR products.	Non-specific bands that occasionally form during extended PCR cycles.	These bands do not indicate mycoplasma contamination. No action required.

** For further information on mycoplasma and mycoplasma detection, please visit www.atcc.org or contact tech@atcc.org or your local distributor.*

800-638-6597 (U.S., Canada, and Puerto Rico)
703-365-2700
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Or contact your local distributor

ATCC™

Universal Mycoplasma Detection Kit

Catalog Number 30-1012K (40 Assays)

Store at -20°C.

This product is intended for laboratory research purposes only. It is not intended for clinical use.

INTRODUCTION

The Universal Mycoplasma Detection Kit offers a quick and sensitive PCR-based test to detect mycoplasma contaminants in cell culture. All components required for the PCR reaction are provided and have been optimized for amplification. High specificity is obtained through the utilization of a proprietary mix of buffers, dNTPs and thermostable polymerase, combined with universal primers that are specific to the 16S rRNA coding region in the mycoplasma genome. DNA originating from other sources, such as tissue samples or E.coli, is not amplified. A touchdown PCR regimen increases sensitivity of the assay, along with enhancing specificity.

The kit detects over 60 species of *Mycoplasma*, *Acholeplasma*, *Spiroplasma* and *Ureaplasma*, including the top eight species most likely to afflict cell cultures: *M. arginini*, *M. fermentans*, *M. hominis*, *M. hyorhinis*, *M. orale*, *M. pirum*, *M. salivarium*, and *A. laidlawii*. Samples that are positive for mycoplasma are easily recognized by a distinct PCR product ranging in size from 434 to 468 bp on an agarose gel.

KIT COMPONENTS

Component	Volume	Composition	Storage
Lysis Buffer	2 mL	Lytic agent + digestive enzymes	-20°C
Universal PCR Mix	0.8 mL	Proprietary mix of buffers, dNTPs, thermostable polymerase	-20°C
Universal Primers	0.1 mL	Proprietary mix of universal forward and reverse primers	-20°C
Sample Lysis Tubes	40 each	2-mL snap cap tubes, tight seal	-20°C
Positive Control	50 µL	1 pg/µL pUC19:: *M.arginini* target in TE	-20°C

QUALITY CONTROL SPECIFICATIONS

Limit of detection (LOD): < 20 genomes of *M.arginini* and *A. laidlawii* are detected in a standard assay. The range of detection varies depending on species, cell type, media and state of cell growth. See www.atcc.org for detection results on over 60 mycoplasma species.

A Certificate of Analysis is available upon request for each lot of the Universal Mycoplasma Detection Kit. The MSDS is available upon request.

EQUIPMENT AND MATERIALS REQUIRED BUT *NOT* INCLUDED IN THE KIT

Microcentrifuge	Thermal cycler and PCR tubes
Heating blocks for microcentrifuge tubes at 37°C and 95°C	Agarose gel electrophoresis apparatus and buffers
Positive-displacement pipette and aerosol-resistant tips	Gel loading dye and DNA stain (ethidium bromide)

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SAMPLE PREPARATION PROTOCOL

Samples should be derived from cell cultures that are 50% to 70% confluent. Use of more than 10^6 cells per sample may inhibit PCR or result in samples that are not homogeneous.

Step	
1	Cell Harvest: A. Suspension cells: Count cells. 10^4 - 10^5 cells are needed for the assay. B. Adherent cells: Scrape the cells into the existing culture media and suspend. **Do not treat cells with trypsin or EDTA** as these agents disrupt mycoplasma.
2	Transfer 1 mL cell suspension (10^4 to 10^5 cells) into the Sample Lysis Tubes and centrifuge at 13,000 rpm for 3 minutes at 4°C. (Note: These tubes were selected for use because they resist opening during the inactivation step 6).
3	Carefully remove and discard the supernatant.
4	Resuspend the cell pellet with 50 µL **Lysis Buffer** by vortexing.
5	Incubate the resuspended cell pellet at 37°C for 15 minutes to lyse the cells and degrade the proteins.
6	Heat the samples at 95°C for 10 minutes to inactivate the protease.
7	Spin down cell debris at 13,000 rpm for 5 minutes at 4°C. Transfer supernatant to a new microcentrifuge tube. Do NOT use the tubes provided with the kit as these are needed for remaining kit assays.
8	Samples are now ready for PCR. If desired, these extracts may be stored at -80°C for up to six months.

PCR PREPARATION PROTOCOL

Precautions for PCR: This kit detects femtogram [(fg) = 10^{-9} µg] quantities of target DNA. Sample preparation, amplification and detection should occur in separate areas and use dedicated equipment. If possible, assemble PCR reactions in a dedicated PCR work station with laminar flow or in a laminar flow hood. It is very important that the positive control does not contaminate other samples. Keep reactions and components capped as much as possible. At a minimum, use pipette tips with hydrophobic filters to avoid cross-contamination with DNA.

Kit Components: Thaw Universal PCR Mix, Universal Primers and Positive Control. Briefly, vortex and spin down components to collect contents at the bottom of the tube prior to opening.

Step	Reaction Setup
1	Prepare a PCR + Primers Mix by combining Universal PCR Mix with Universal Primers:

Component	Vol. per Assay	Vol. for 5 Assays	Vol. for 10 Assays	Vol. for 20 Assays	Vol. for 40 Assays
Universal PCR Mix	20 µL	100 µL	200 µL	400 µL	800 µL
Universal Primers	2.5 µL	12.5 µL	25 µL	50 µL	100 µL
TOTAL Volume	22.5 µL	112.5 µL	225 µL	450 µL	900 µL

Note on number of assays to prepare: The PCR + primers mix is needed for positive and negative controls (2 assays). We also suggest that a positive control + test sample assay is prepared for each sample to confirm that the cell lysate (sample) does not inhibit PCR. It is recommended that test samples are prepared in duplicate.

ATCC
Universal Mycoplasma Detection Kit
Instruction Sheet

2 Prepare the reaction mixtures in PCR tubes as follows:

Component	Test Samples	Positive Control	Positive Control + Test Sample*	Negative Control
Universal PCR Mix + Primers Mix	22.5 µL	22.5 µL	22.5 µL	22.5 µL
Test Sample	2.5 µL	----	2.5 µL	----
Positive Control	----	2.5 µL	1.0 µL	----
H_2O or TE	----	----	----	2.5 µL
TOTAL volume	25 µL	25 µL	26 µL	25 µL

*We recommend that this control is prepared for each test sample.
Store the remaining extract for each test sample at -80°C in the event further testing is needed.

3 Mix gently by pipetting the reaction mixes up and down a few times. Cap tubes and centrifuge briefly to bring fluid to the bottom of the tube.

PCR Amplification Procedure

4 Place the tubes in a thermal cycler.

5 Use the following parameters for PCR:

Step 1	**Initial Denaturation: 94°C for 1.5 min**		
Step 2	**Touchdown PCR Parameters:**		
	Temperature °C	**Time (seconds)**	**Cycles**
Denaturation	94	30	20
Annealing	70 → 60.5*	30	
Elongation	72	45	
***Temperature decreases 0.5°C per cycle** (e.g., 70°C for 1 cycle, 69.5°C for 1 cycle, etc., to 60.5°C for 1 cycle).			
Step 3	**Continue cycling at a constant Annealing Temp.:**		
Denaturation	94	30	12**
Annealing	60	30	
Elongation	72	45	
Step 4	**Final Elongation:**	**72°C for 4 min 4°C on HOLD**	
** To increase the sensitivity of the assay, perform 20 PCR cycles at this step. This increased level of sensitivity requires special precautions to minimize contamination with airborne environmental bacterial DNA that can be amplified with mycoplasma-specific primers.			