For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

ISO 9001/14001 Certified Company

M-Solution™ 1-2 Antibiotics
(100x concentrated)

Cat. No. 21081 10 ml / each

DESCRIPTION

Mycoplasma are common and serious contaminants of cell cultures. It has been shown that 30-87% of cell cultures are infected with mycoplasma. Many mycoplasma contaminations, particularly in continuous cell lines, grow slowly and do not destroy host cells but are still able to affect various parameters, leading to unreliable or false results. These effects include changes in metabolism, growth, viability, DNA, RNA, protein synthesis, and morphology etc. Testing for mycoplasma infection is essential quality control tool to assure accurate research and reliable biotechnological products. M-Solution™ 1-2 Antibiotics (100x) are a combination of antibiotics, which have been shown to be effective in the elimination of mycoplasma species that account for 90% of the contamination found in cell cultures. M-Solution™ 1-2 Antibiotics (100x) are antibiotic formulations designed to treat Mycoplasma infected cell cultures. M-Solution™ 1 Antibiotic is a solution based on the antibiotic Tiamutin. M-Solution™ 2 Antibiotic is a solution based on the antibiotic Minocycline, which is a member of the Tetracycline group. These two antibiotics have been shown to be effective in eliminating the mycoplasma species frequently present in contaminated cell cultures. Furthermore, the mycoplasmas do not develop resistance to these antibiotics – a common occurrence with other antibiotic treatment methods. The two antibiotic solutions are generally used sequentially, in combination. The two antibiotic solutions are used sequentially over a period of 1 week, and the cycle is repeated 2-3 times as required. The treatment is quite easy to perform and when used according to the following protocol, no cytotoxic effects will occur.

KIT CONTENTS and STORAGE

- M-Solution™ 1-2 Antibiotics (100x) is a sterile, ready-to-use solution. Store the original container at -20°C.
- Always avoid multiple freeze-thaw cycles or exposure to frequent temperature changes.

Kit Contents	Amount
M-Solution™ 1 Antibiotic (100x)	10 ml
M-Solution™ 2 Antibiotic (100x)	10 ml

CHARACTERISTICS

- **Highly Effective**

M-Solution™ 1-2 Antibiotics are effective against mycoplasma species usually encountered as contaminants in cell cultures.

- **No cytotoxic effect**

Kills mycoplasmas, but safe for cells; M-Solution™ 1-2 Antibiotics are the first biological reagent that eliminates mycoplasmas by killing them, and not just by inhibiting growth. The cytotoxicity of M-Solution™ 1-2 Antibiotics is comparably low.

- **Ease-to-use solutions**

M-Solution™ 1-2 Antibiotics have been shown to be effective with only two treatments, where mycoplasmas are permanently destroyed within 3 weeks.

SUPPLEMENTAL REQUIREMENTS

- Standard cell culture equipment (e.g. Humidified 37℃ incubator with 5% (v/v) CO_2 or clean bench etc..)
- Appropriate culture media (e.g. mycoplasma free RPMI 1640 media and FBS)
- Sterile plastic wear
- Mycoplasma detection system to verify the elimination success by using the "e-Myco™ Mycoplasma PCR Detection Kit (iNtRON, Cat. No. 25233)"

CONSIDERATION BEFORE USE

1) M-Solution™ 1-2 Antibiotics (100x) could thaw and freeze 2-3 times. However, it is recommended to make aliquots after the first thaw and freeze the smaller quantities for future use. This will help minimize the number of freeze thaw cycles.
2) M-Solution™ 1-2 Antibiotics should be used for the treatment period only. It should NOT be added to the freezing media when freezing the cells.
3) The cells may be recontaminated again after a successful treatment from several sources (humans, serum, animal proteins, other cells, etc). After successful treatment the cells should be handled properly to prevent contamination. After treatment, the customer may use any type of medium. Please note that M-Solution™ 1-2 Antibiotics are not intended for a routine use in cell culture.

PROTOCOL

When used according to the following instructions, no cytotoxic effects will occur.
All process should be performed under clean bench.

1. Thaw M-Solution™ 1-2 Antibiotics.
Note : M-Solution™ 1-2 Antibiotics may be refrozen 2-3 times. However, it is recommen -ded to make aliquots after the first thaw and freeze the smaller quantities for future use. This will help minimize the number of freeze thaw cycles.

2. Prepare each culture media by adding the M-Solution™ 1 Antibiotic or 2 Antibiotic (100x) separately as 1/100 concentration. And then M-Solution™ 2 Antibiotic should be stored at 4°C until use.
Note 1 : DO NOT USE TWO SOLUTIONS TOGETHER IN SAME CULTURE MEDIA.
Note 2 : For example, add 1 ml of M-Solution™ 1 Antibiotic to 100 ml of fresh medium and other 100 ml of fresh medium should be added 1 ml of M-Solution™ 2 Antibiotic. The each medium contained M-Solution™ 1 or 2 Antibiotic should be stored at 4℃.
Note 3 : M-Solution™ 1 Antibiotic is a solution based on the antibiotic Tiamutin(Tiamulin) which inhibits protein synthesis of prokaryotes. Also, M-solution™ 2 Antibiotic is base on the antibiotic Monocycline (Tetracyclin group) which inhibits protein synthesis of broad range of bacteria by preventing binding of aminoacyl-tRNA to ribosome. Therefore, any other antibiotics were not needed in M-Solution™ treatment.

3. Harvest the cultured cells (in the case of adherent cells by use of trypsin/EDTA [0.25%/0.02%]). Suspended or adherent cells of various cell lines (for example, K562 Human immortalised myelogenous leukaemia cells and NIH 3T3 mouse embryonic fibroblast cells) which were contaminated with mycoplasmas of unidentified specificity were inoculated at a concentration of 10^5 Cell / ml and volume of 10~15 ml of fresh medium contain M-Solution™ 1 Antibiotic into 75 cm^3 of cell culture flask.
Note : The growth condition of cell line depends on the mass of inoculum.

4. Incubate the cell for 4 days in a humidified 37℃ incubator with 5% (v/v) CO_2.

5. After 4 days, harvest the cultured cell, then inoculate the cells again at 10^5 Cells / ml in fresh medium containing the M-Solution™ 2 Antibiotic.

6. Incubate the cell for 3 days in a humidified 37℃ incubator with 5% (v/v) CO_2.

7. The above, together, are considered one treatment cycle. Repeat as in the step 3-6 once again.
Note : To surely removing mycoplasma, repeat as in the step 3-6 one more time.

8. After the above treatment cycle, incubate the cells in media without M-Solution™ 1-2 Antibiotics.
Note : The period for incubating the cell in media without these antibiotics is recommende -d for 14 days during fresh media is changed.

9. During this process, estimate the level of elimination of mycoplasma by using *e*-Myco™ Mycopasma PCR Detection Kit (iNtRON, Cat. No. 25233).
Note 1 : In order to estimate disinfection of mycoplasma more fast and sensitive, *e*-Myco™ Mycoplasma PCR Detection Kit (iNtRON, Cat. No. 25233) is recommended to use, strongly.
Note 2 : To avoid inadvertent contamination of clean cell lines, mycoplasma testing should be segregated to a laboratory not used for general cell culture work.
Note 3 : If the result of mycoplasma is positive, the cultured cells should be treated with M-Solution™ 1-2 Antibiotic repeatedly.

TECHNICAL INFORMATION

EXPERIMENTAL DATA

● ***Estimate the level of elimination of mycoplasma***

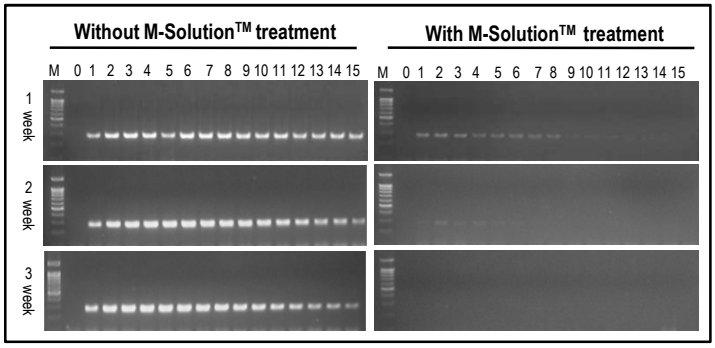

Fig. 1. Result for the various cell number

To estimate the level of elimination of mycoplasma for the various cell number, it was performed with *e*-Myco™ Mycoplasma PCR Detection Kit (iNtRON, Cat. No. 25233).

Lane M, 100 bp Ladder DNA Marker (Cat. No. 24022); **lane 0,** Negative control; **lane 1,** $2x10^5$ cells; **lane 2,** $1x10^5$ cells; **lane 3,** $5x10^4$ cells; **lane 4,** $2.5x10^4$ cells; **lane 5,** $1.25x10^4$ cells; **lane 6,** $6.25x10^3$ cells; **lane 7,** $3.125x10^3$ cells; **lane 8,** $1.56x10^3$ cells; **lane 9,** $7.8x10^2$ cells; **lane 10,** $3.9x10^2$ cells; **lane 11,** $1.9x10^2$ cells; **lane 12,** 98 cells; **lane 13,** 49 cells; **lane 14,** 24 cells; **lane 15,** 12 cells

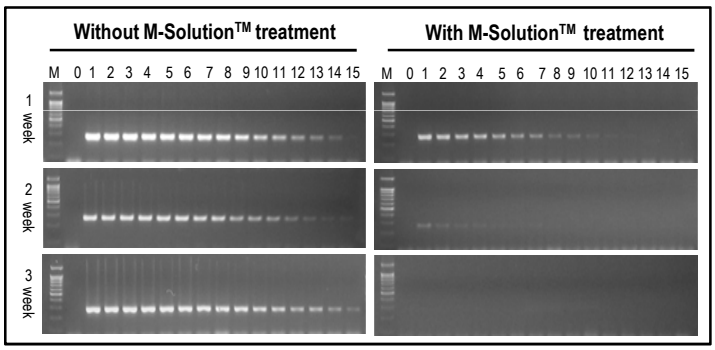

Fig. 2. Result for the various concentration of template DNA

To estimate the level of elimination of mycoplasma for the various concentration of template DNA, it was performed with *e*-Myco™ Mycoplasma PCR Detection Kit (iNtRON, Cat. No. 25233).

Lane M, 100 bp Ladder DNA Marker (Cat. No. 24022), **lane 0,** Negative control; **lane 1,** 100ng; **lane 2,** 50ng; **lane 3,** 25ng; **lane 4,** 12.5ng; **lane 5,** 6.3ng; **lane 6,** 3.2ng ; **lane 7,** 1.6ng; **lane 8,** 800pg; **lane 9,** 400pg; **lane 10,** 200pg; **lane 11,** 100pg; **lane 12,** 50pg; **lane 13,** 25pg; **lane 14,** 12.5pg; **lane 15,** 6.3pg

RELATED PRODUCT

● ***e*-Myco™ Mycoplasma PCR Detection Kit (iNtRON, Cat. No. 25233, 96 Tests)**

e-Myco™ Mycoplasma PCR Detection Kit is primer sets designed to detect the presence of mycoplasma which might contaminate in biological materials such as cultured cells. Conventional techniques used to detect mycoplasma involve culturing samples on selective media, which needs at least a week to obtain results. Whereas, by performing PCR with this primer sets based on an conserved 16S rRNA, detection results are obtained in a few hours. As the presence of contaminant mycoplasma can be easily detected by only verifying the bands of amplified DNA fragments in electrophoresis, there is no need to prepare probes labeled with radioisotope, etc.

[CHARACTERISTICS]

- ***Premix type***
  : This e-Myco™ kit contains all components for PCR reaction. You can add just a template DNA or samples.
- ***Broad Species Detection***
  : You can detect the five common cell-culture-infecting species of mycoplasma, and also other various species of mycoplasma (See the table below).
- ***Species Determination***
  : You can determine the species of mycoplasma by sequencing from the amplified PCR products.

[Table 1. Mycoplasma Species Detected by e-Myco™ Kit]

M. species	*Origin Type*	*Primer Type*	*PCR Size*
A. laidlawii	A/E	A	I
M. adleri	J	M2	III
M. agalactiae	F	M2	III
M. alkalescenns	E	M5	III
M. anseris	K	M5	II
M. arginini	A/B	M5	III
M. arthritidis	H	M5	III
M. auris	F	M5	III
M. bovigenitalium	E	M2	III
M. bovirhinis	E	M2	IV
M. bovis	E	M2	III
M. buccale	B	M5	II
M. californicum	E	M2	II
M. canadense	E	M5	III
M. caviae	L	M2	III
M. citelli	M	M2	I
M. cloacale	N	M5	II
M. columbinasale	C	M2	II
M. columbinum	C	M2	III
M. equirhinis	G	M4	II
M. falconis	C	M2	IV
M. faucium	A	M1	I
M. felifaucium	O	M2	II
M. fermentans	A	M2	III
M. gallinarum	C	M2	III
M. gateae	P	M5	III
M. hominis	A	M6	II
M. hyorhinis	A/D	M2	V
M. hyosynoviae	P	M5	II
M. iguanae	V	M4	III
M. indiense	Q	M3	II
M. iners	C	M2	II
M. leopharyngis	R	M2	II
M. maculosum	P	M2	II
M. meleagridis	C	M2	II
M. moatsii	S	M2	III
M. mustelae	T	M2	I
M. opalescens	P	M2	III
M. orale	A	M3	II
M. oxoniensis	U	M2	I
M. penetrans	B	M7	VI
M. primatum	Q	M2	III
M. pulmonis	H	M5	IV
M. salivarium	A	M5	II
M. spermatophilum	B	M2	II
M. sualvi	P	M2	III
M. subdolum	G	M5	III
M. synoviae	C	M2	I
M. verecundum	E	M2	I

More information are available from our website: http://www.intronbio.com.