



## TECHNICAL INFORMATION

Catalog Number: 3050044

### Mycoplasma Removal Agent (MRA)

**Unit Size:** 5 ml (50 ug/ml)

#### 1. Introduction

Mycoplasma Removal Agent (MRA) has been developed by Dainippon Pharmaceutical Co., Ltd. in Japan for cell culture. This agent has been shown to be effective in the elimination of various types of mycoplasma from contaminated cultures. MRA can be used to prevent recontamination of cured cultures by mycoplasma.

The product contains 50 ug of 4-oxo-quinoline-3-carboxylic acid derivative in 1 ml of water.

#### 2. Features

1. MRA shows a strong anti-mycoplasma activity against various types of mycoplasma.
2. If cells are treated with MRA, recontamination of that culture with the original mycoplasma is not detected while preventative doses of MRA are in use.
3. MRA can be used very conveniently. Simply add to cell cultures contaminated by mycoplasma and incubate for a week.
4. As a preventative measure, MRA can be used to avoid mycoplasma contamination. MRA should not be used as a substitute for good cell culture techniques.

#### 3. Procedure

1. Add MRA to cell cultures contaminated by mycoplasma at a concentration of 0.5 ug/ml and incubate for a week. (Add 0.1 ml of MRA in the case of 10 ml of media in a 25 cm<sup>2</sup> flask.)
2. Use a medium containing MRA at the same concentration for media replacement or culture transfer (passage).
3. Transfer the cell cultures several times without MRA and confirm that regrowth of the contaminating mycoplasma has not occurred.
4. A Mycoplasma detection kit (such as MP's Mycoplasma Hoechst Stain Kit, catalog number 3030000, or Immu-Mark Myco-Test Kit, catalog number 3020000) is recommended for the detection of contamination.
5. If there is a concern about the presence of mycoplasma in serum or trypsin, contamination of the cell cultures exposed to these products can be prevented by adding MRA at a concentration of 0.1 ug/ml to the media.

#### 4. Storage

MRA is stable at room temperature. Protect from light to prevent decomposition.

#### 5. Caution Upon Use

1. MRA is a research reagent and must be used only as a mycoplasma removal agent in cell cultures. MRA is a synthetic molecule. Good laboratory practices should be observed when handling the product.
2. The recommended concentration for use is 0.5 ug/ml. Concentration may be raised up to 1 ug/ml only when the recommended concentration is ineffective in removing the mycoplasma. For preventative purposes, MRA can be used at 0.1 ug/ml.
3. The cytotoxicity of MRA is low and cell toxicity is rare when used at the recommended concentration. For

specific function of any cell, however, it is recommended that the retention of desired cellular characteristics be confirmed after treatment.

## 6. Sample Data

Note that the level of infection, cell type and mycoplasma strains may influence specific results. Each researcher should use the sample data as a guide from which to determine the effective MRA concentration needed with their specific cell line and mycoplasma strain.

### 1. Data on mycoplasma removal effect - spontaneous infection.

	Concentration of MRA (ug/ml)	Duration of cultures (days)			
		0	7	14	21
Human-derived cell-A (Human melanoma)	0.39	+	-	-	-
	0.2	+	-	-	-
	0.1	+	-	+	+
	0	+	+	+	+
Human-derived cell-B (Human lung carcinoma)	0.78	+	-	-	-
	0.39	+	-	-	-
	0.2	+	-	-	-
	0.1	+	+	+	+
	0	+	+	+	+

+ = Mycoplasma positive

- = Mycoplasma negative

Duration treated with MRA: 7 days

### 2. Efficacy comparison of MRA and pharmaceuticals on the market.

	MRA		Tiamulin		Minocycline	
	MIC*	MMC**	MIC	MMC	MIC	MMC
M. orale CH-19299	0.05	0.1	0.0031	3.13	0.05	25
M. arginini G-230	0.1	0.2	0.0063	12.5	0.2	>100
M. hyorhinis BST-7	0.05	0.1	0.0031	0.39	0.0031	0.39
A. laidlawii PG-8	0.0125	0.025	0.05	>100	0.05	>100
MMC/MIC	2		128	> 2048	512	> 2048

M. = Mycoplasma A. = Acholeplasma

\* = Minimum inhibitory concentration (ug/ml) \*\* = Minimum mycoplasmacidal concentration (ug/ml)

### 3. MIC of MRA to other mycoplasma (ug/ml)

Species	MIC
Mycoplasma fermentans PG-18	0.0125
Mycoplasma salivarium PG-20	0.1
Mycoplasma hominis PG-21	0.1
Mycoplasma buccale CH-20247	0.025

This product has been expressly manufactured by Dainippon Pharmaceutical Co., Ltd. in co-operation with MP Biomedicals, Inc.