IMMUNOLOGICAL SCIENCES Product Data Sheet

ISK-X15-005 Mycoplasma Removal Agent (MRA)

Unit Size: 5ml (1000X)

Storage

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

1. Introduction

Mycoplasma Removal Agent (MRA) has been developed by Immunological Sciences for cell culture. This agent has been shown to be effective in the elimination of various types of mycoplasma from contaminated cultures. MRA canbe used to prevent recontamination of cured cultures by mycoplasma.

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 preventativedoses 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 substitutefor good cell culture techniques.

3. Procedure

a) Add MRA to cell cultures contaminated by mycoplasma at a concentration of1X and incubate for a week. (Add 10uL of MRA in the case of 10 ml of media in a 25 cm^2 flask.)
b) Use a medium containing MRA at the same concentration for media replacement or culture transfer (passage).
c) Transfer the cell cultures several times without MRA and confirm that regrowth of the contaminating mycoplasma has notoccurred.
d) A Mycoplasma detection kit (such as Mycoplasma Hoechst Stain Kit) is recommended for the detection of contamination.
e) If there is a concern about the presence of mycoplasma in serum or trypsin, contamination of the cell cultures exposed tothese products can be prevented by adding MRA at a concentration of 0.2X to the media.

4. 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 syntheticmolecule. Good laboratory practices should be observed when handling the product.
2. The recommended concentration for use is 1x . Concentration may be raised up to 2x only when the recommended concentration is ineffective in removing the mycoplasma. For preventative purposes, MRA can be used at 0.2x.
3. The cytotoxicity of MRA is low and cell toxicity is rare when used at the recommended concentration. For specific function ofany cell, however, it is recommended that the retention of desired cellular characteristics be confirmed after treatment.

5. Sample Data

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

For Research use only

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1. Data on mycoplasma removal effect - spontaneous infection.

	Concentration ofMRA (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