

MycoZap™ mycoplasma elimination reagent

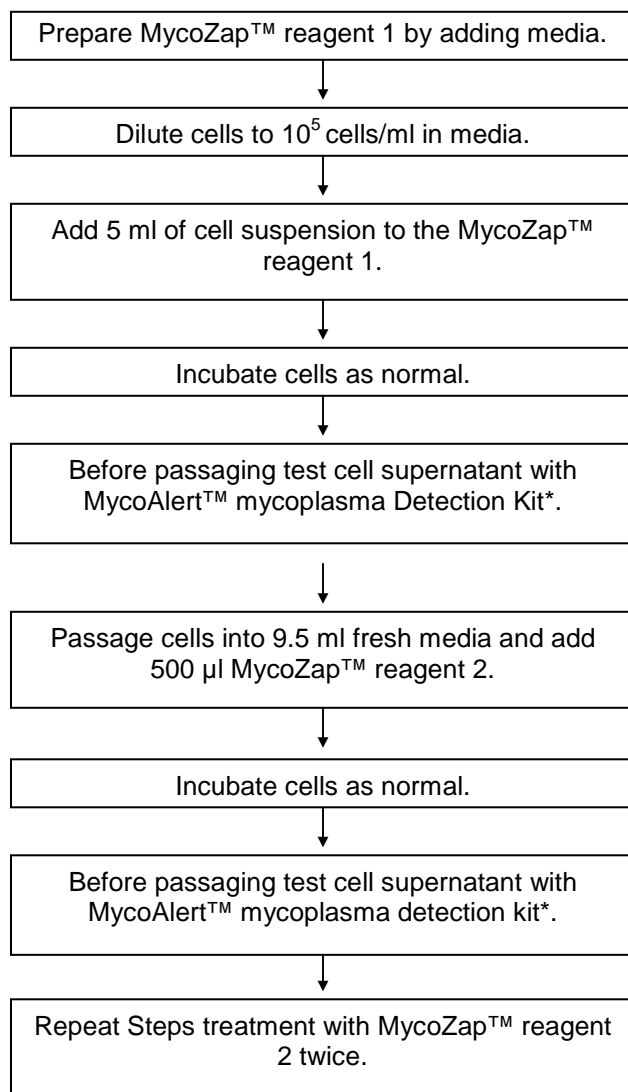
Instructions for use

Safety

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

1. MycoZap™ assay procedure outline

(For detailed assay protocol see section 5)



*Sold separately.

All trademarks herein are marks of Lonza Group or its subsidiaries. Developed by Lonza Nottingham, Ltd.

2. Kit contents & storage

| LT07-818 | 1 treatment |
|--------------------|-----------------------|
| MycoZap™ reagent 1 | 1 x 0.5 ml (LT27-280) |
| MycoZap™ reagent 2 | 1 x 1.5 ml (LT27-281) |

| LT07-918 | 5 treatments |
|--------------------|-----------------------|
| MycoZap™ reagent 1 | 1 x 2.5 ml (LT27-282) |
| MycoZap™ reagent 2 | 1 x 7.5 ml (LT27-283) |

The kit contains all the required reagents to perform the elimination process. MycoZap™ reagent 1 and 2 are provided ready for use.

The kit should be stored at 2°C-8°C. See kit label for expiration date of the whole kit. See bottle labels for expiration dates of individual components.

Related products

| | |
|-------------------------------------|--------------|
| MycoAlert™ mycoplasma detection kit | |
| LT07-118 | 10 Test Kit |
| LT07-218 | 25 Test Kit |
| LT07-418 | 50 Test Kit |
| LT07-318 | 100 Test Kit |

3. Intended use

NOTE: Lonza strongly recommends that cell cultures with mycoplasma contamination are discarded and fresh stocks obtained. Infection of cells with mycoplasma can affect every known process in the cell and it is not known if all functions return upon the elimination of the infection. In cases where fresh stocks cannot be obtained, MycoZap™ reagent offers a reliable method of mycoplasma elimination.

Mycoplasmas are the smallest and simplest prokaryotes and they depend on their hosts for many nutrients due to their limited biosynthetic capabilities. They have long been recognized as common contaminants of cell lines in continuous culture but their presence may go undetected for

months. As the mycoplasma competes with the cells for the nutrients in culture media, one of the first signs is a reduction in the rate of cell proliferation and slight changes in cellular responses including gene expression.

It is reported that between 5% - 35% of all cells in continuous culture are contaminated with mycoplasma. An infection can have serious consequences affecting the reliability, reproducibility and consistency of results obtained from these cultures. Mycoplasma infections can be easily spread within the culture environment therefore regular testing of cell lines using assays such as Lonza's mycoAlert™ mycoplasma detection Kit should be incorporated into the culturing regime. In cases where contamination has occurred, and the sample absolutely cannot be discarded, MycoZap™ reagent has been designed to eliminate mycoplasma with minimal toxic effects on the cells.

4. Elimination principle

MycoZap™ reagent eliminates mycoplasma by using a combination of antibiotic and antimetabolic agents. This approach allows for a highly reliable and definite elimination of mycoplasma that cannot be achieved by the use of antibiotics alone. MycoZap™ can be used to eradicate mollicutes, including *mycoplasma*, *acholeplasma*, *spiroplasma* and *entomoplasma* species in cell cultures.

5. Protocol

NOTE: MycoZap™ reagent 1 treatment must be conducted in media containing a **maximum of 5% (v/v) serum**. The subsequent treatment with MycoZap™ reagent 2 can be conducted in media containing normal amounts of serum. For the most effective treatment it is important that a single cell suspension is used.

1. Add 4.5 ml of supplemented culture media (containing a maximum of 5% (v/v) fetal calf serum) to a 25 cm² culture flask.
2. Add 500 µl of **MycoZap™ reagent 1** to the media and swirl gently to mix.
3. Prepare cell suspension (by trypsinization if required) to a cell density of 10⁵ cells/ml in supplemented culture media (containing <5% (v/v) fetal calf serum).
4. Add 5 ml of the cell suspension to the MycoZap™/media mix.
5. Incubate flask as normal for 2-6 days depending on cell proliferation rate.
6. Prior to passage, remove 2 ml of the cell culture supernatant for testing with MycoAlert™ assay.
7. Passage cells as normal into 9.5 ml of fresh media.
8. Add 500µl of **MycoZap™ reagent 2** to the cells

All trademarks herein are marks of Lonza Group or its subsidiaries. Developed by Lonza Nottingham, Ltd.

- and swirl gently to mix.
9. Incubate flask as normal for 2-6 days depending on cell proliferation rate.
10. Repeat steps 6-9 twice.
11. After the end of the third treatment with MycoZap™ reagent 2, the cells should be free of infection.
12. Confirm the success of the treatment by removing 2 ml of the cell culture supernatant for testing with MycoAlert™ assay.

NOTE: Cultures should be tested with the MycoAlert™ mycoplasma detection kit at regular intervals for 4-6 weeks after mycoplasma elimination to ensure fresh infections do not arise.

THESE PRODUCTS ARE FOR RESEARCH USE ONLY.

Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

© 2011 Lonza Walkersville, Inc.

All rights reserved.