

CELLshipper® Mycoplasma Detection Kit



Protocol

The CELLshipper® slide is specially designed and coated to support fixation of adherent or nonadherent cell cultures for DNA fluorochrome staining. The following simple protocol is the recommended fixation procedure for this product.

(Please click [here](#) to view the product data sheet for the M-100 CELLshipper® Mycoplasma Detection Kit)

Materials:

- CELLshipper® slide in plastic fixation/staining chamber (provided by Bionique)
- Test Article: Cell culture (recommended subculture ≥ 3 times without antibiotics prior to testing)
- Pipettes
- Cell scraper (recommended but 1 mL plastic pipette can be used)
- Carnoy's fixative (1 part glacial acetic acid plus 3 parts reagent grade methanol)
 - **PREPARE FRESH FOR EACH USE.**
 - *Caution:* Fixative is toxic and corrosive.
 - Do not use acetone, chloroform or similar reagents with the CELLshipper®.

Procedure:

1. **Adherent cells** - Remove most of the medium, leaving approx. 3 mL in a T 25 or 5 mL in a T 75 flask. **Scrape** the cells from the substrate and break up the cell clumps by forcibly pipetting. Use **nearly** confluent cells in conditioned medium 24-72 hours after the last subculture.
Nonadherent cells - Withdraw 2 mL of a cell suspension with $\geq 80\%$ viability that has not been re-fed in the last 24-72 hours.
2. Remove the CELLshipper® slide from the plastic chamber and place it in a flat level surface (preferably in a petri dish). Pencil sample identification data onto the frosted end of the slide. Carefully pipette 2 mL of the freshly prepared cell suspension onto the large clear zone within the orange border.
3. Incubate undisturbed (preferably in a covered petri dish) at room temperature ($\geq 22^\circ \text{C}$) for 60 minutes. The glass slide is treated to enhance cell attachment and a sufficient number of cells will adhere if incubation parameters are followed.
4. **DO NOT ALLOW THE GLASS SLIDE TO DRY.** Prepare 40 mL of fresh Carnoy's fixative and add 20 mL to the plastic chamber oriented vertically (cap up or on top). Remove the growth medium and quickly, insert the wet CELLshipper® slide into the last slot (nearest the top hinge) with cells and orange border facing in to the plastic chamber (1 slide per chamber). Fix for 5 minutes at room temperature.
5. Remove initial fixative; add 20 mL of Carnoy's fixative and fix cells for an additional **10 minutes**.
6. Remove fixative. Allow CELLshipper slide and plastic chamber to **air dry completely** before closing the plastic chamber (1 slide per chamber)
7. **Fill out** the enclosed information sheet **completely** and enclose with the dry CELLshipper® slide in its' individual plastic chamber in the cardboard mailer provided.
8. Seal cardboard mailer, record return address; affix proper **first class postage** and mail. For **faster** delivery, ship via priority mail or courier service.

BIONIQUE will process sample and provide results within 48 hours of receipt.

Optional:

CELLshipper® slide preparation and sample fixation can be performed by our technicians with the M-175 assay.
(see M-175 assay for additional details)

References:

1. McGarrity, G.J., Steiner, T., and Vanaman, V. 1983. Detection of mycoplasmal infection of cell cultures by DNA fluorochrome staining. In *Methods of Mycoplasma*, vol. II (Tully and Razin, eds.) Academic Press, New York, pp. 183-190.
2. Del Guidice, R.A., Hopps, H.E. 1977. Microbiological methods and fluorescent microscopy for direct demonstration of mycoplasma infection in cell cultures. In *Mycoplasma Infection of Cell Cultures* (McGarrity, Murphy and Nichols, eds.) Plenum Press, New York pp. 57-69.
3. McGarrity, G.J., Sarama, J. and Vanaman, V. 1979. Factors influencing microbiological detection in cell cultures. *In Vitro* 15:73-81.
4. Chen, T.R. 1977. In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. *Exp. Cell Res.* 104:255-262.
5. Lincoln, C.K., Gabridge, M.G. 1998. Cell Culture Contamination: Sources, Consequences, Prevention and Elimination. *Methods In Cell Biology*, Vol. 57 Chapter 4, pp. 49-65. Academic Press, New York.