Mycoplasma Detection Kit

Cat: CA1080
Storage: 2-8 °C, valid for 1 year, Hoechst working fluid needs to be stored away from light.
Product contents:

Ingredients	100T	200T
Hoechst 33258 working fluid	100mL	2×100mL
Fixing liquid	100mL	2×100mL
Anti-fluorescence attenuation sealing solution	20mL	40mL

Product introduction:

This kit uses fluorescent dye (bisbenzimide,Hoechst 33258) to detect mycoplasma contamination. This dye binds to the A-T-enriched region of DNA, which can be detected by staining mycoplasma DNA because of its high A-T content (55%-80%). After staining cells contaminated with mycoplasma, many homogeneous fluorescent dots can be seen around the cells, that is, the DNA stain of mycoplasma, indicating the presence of mycoplasma contamination. Hoechst 33258 had a maximum excitation wavelength of 346nm and a maximum emission wavelength of 460nm. When combined with double-stranded DNA, Hoechst 33258 has a maximum excitation wavelength of 352nm and a maximum emission wavelength of 461nm.

Protocols (only for reference) :

Adherent cells:

1. The tested cells were inoculated in a sterile 6-well cell culture plate with a inoculation density of 1-2×10^4. At the same time, the same kind of cells without normal mycoplasma infection were inoculated as negative control.
2. 5 days later, the culture medium was sucked, then 1mL of fixing solution was added, and the cells were left standing for 20min.
3. Suck the fixing solution and let it dry.
4. Add 1mL of Hoechst33258 working liquid into each well (Hoechst33258 working liquid must cover all tested cells) and place at 37℃ away from light for 15-20min or at room temperature for 20-30min.
5. Absorb the Hoechst 33258 working liquid, add 2mL sterilized ultra-pure water to wash three times, and directly air dry. After air drying, add a drop of sealing liquid, and cover with a cover glass.
6. Observe with fluorescence microscope. Use ultraviolet excitation light excitation to observe whether there are blue fluorescent dots or beaded fluorescent dots around the cell.

Suspension cells:

1. Collect the cells to be detected at 1500rpm for 5min.
2. Smear the collected cells on a slide, add 1mL fixing solution, and stand for 20min.
3. Blot the fixing solution and let dry.
4. Add 1mL of Hoechst33258 working liquid into each well (Hoechst33258 working liquid must cover all tested cells) and place at 37℃ away from light for 15-20min or at room temperature for 20-30min.
5. Absorb the Hoechst 33258 working liquid, wash the slide with sterile water 3 times, and air dry directly. After air drying, add a drop of the sealing solution and cover with a cover glass.
6. Fluorescence microscope observation. Use ultraviolet excitation light excitation to observe whether there are blue fluorescent dots or beaded fluorescent dots around the cell.

Result judgment (reference) :

Negative: Only the cell nucleus showed yellowish-green fluorescence.

Positive: Except cells, a large number of uniformly sized fluorescent colored particles can be seen around the cells.

Notes:

1. Hoechst working fluid is harmful to human body, please pay attention to protection.
2. fixed liquid has pungent smell, it is recommended to fix in the fume hood.
3. mycoplasma detection, it is best to use antibioticless culture medium 2-3 generations, so it is easy to avoid false negative results.
4. When this kit is used for 6-well plate detection, it can carry out 50 detection reactions.
5. To detect mycoplasma contamination, efficient Vero cells of mycoplasma can be used, which can improve the detection sensitivity, and the tested sample will be inoculated in Vero cells for detection.

Related products:

P2100 10×polylysine
10491 anti-fluorescence attenuation sealer
C0020 Hoechst 33258 Dyeing Solution (Ready-to-use)