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Mycoplasma Detection Kit

Cat Number: CA1080

Storage: 2-8℃, valid for 1years, Hoechst solution should be stored in a dark place.

Kit components

	100T	200T
Hoechst 33258 solution	100ml	2×100ml
Fixative solution	100ml	2×100ml
Mounting liquid	20ml	2×20ml

Product Description

The Mycoplasma Detection Kit is using fluorescent dyes (bisbenzimide, Hoechst33258) to detect mycoplasma infections. The dye could binds to the A-T enriched region of DNA, because of the high content of A-T in the DNA of mycoplasma (55%~80%) it can be stained and detected. After staining, around the cells infected by mycoplasma can be seen many small and uniform fluorescent dots, that is, the DNA stain of mycoplasma, which shows that there are mycoplasma infections.

The maximum excitation wavelength of Hoechst33258 is 346nm, and the maximum emission wavelength is 460nm. After combined with double stranded DNA the maximum excitation wavelength of Hoechst33258 is 352nm, and the maximum emission wavelength is 461nm.

Procedure

I. Adherent Cell:

1. The tested cells were inoculated in a sterile 6 well cell culture plate (density 1-2×10^4). At the same time, inoculated with the same kind of uninfected cells, as negative control.
2. 5 days later, remove the culture medium, add 1ml fixative solution, and rest for 20minutes.
3. Remove fixative buffer, dry out.
4. Add 1ml Hoechst33258 solution(Covering all the cells to be tested) in every well, rest in a dark place, and incubate at 37℃ for 15-20min or room temperature 20～30min.
5. Remove Hoechst33258 solution. Add 2ml sterilized ultra pure water washing three times. After dry out add a drop of mounting liquid and cover with a cover slide.
6. Fluorescence microscope observations. Excitation with UV excitation , observe whether there are blue fluorescent dots or beaded fluorescent dots around the cells.

II. Suspension Cell:

1. Collect the cells to be tested. Centrifuge the sample at1500rpm for 5min.
2. Smear the collected cells on slide, add 1ml fixative solution, and rest for 20minutes.
3. Remove fixative buffer, dry out.
4. Add 1ml Hoechst33258 solution(Covering all the cells to be tested)in every well, rest in a dark place, and incubate at 37℃ for 15-20min or room temperature 20～30min.
5. Remove Hoechst33258 solution. Add 2ml sterilized ultra pure water washing three times. After dry out add a drop of mounting liquid and cover with a cover slide.
6. Fluorescence microscope observations. Excitation with UV excitation, observe whether there are blue fluorescent dots or beaded fluorescent dots around the cells.

Result judgment(reference)

1.**Negative:** Only cell nucleus showed yellow green fluorescence.

2.**Positive:** In addition to the cell, cells are surrounded by a large number of uniformly sized fluorescent colored particles.

Notes:

1. Hoechst solution is harmful to the human body, please pay attention to the protection.
2. Fixative solution have a pungent odor, recommended for operation in the fume hood.
3. It is best to cultivate the culture medium without antibiotics for 2~3 generations. It is easy to avoid false-negative results.
4. When the kit is used for 6-well plate detection, it can carry out 50 detection reactions.
5. When detection of mycoplasma infections, can use a highly efficient Vero cells, which could improve the detection sensitivity, will be detected in the samples inoculated with Vero cells for detection. (The samples were inoculated into Vero cells for detection.)

Related Products:

P2100 Poly-L-lysine Solution,10×

S2100 Mounting Medium, antifading

C0020 Hoechst 33258 Stain solution(ready-to-use)