Cell Culture Contamination Detection Kit

Cat #: orb1566775 (manual)

Size: 20 tests

Product Composition

Reagent name	Specifications
Calcofluor White M2R	10µl
MycoLight Green JJ98	10µl
WGA-iFluor 594	50µl
Manual	1 copy

Product Introduction

The Cell Culture Contamination Detection Kit provides a simple and effective method for detecting microbial contamination during the cell culture process. The cell culture contamination detection kit is not only used to detect the presence of pollutants, but also to identify the types of pollutants, including yeast (and other fungi), Gram-positive bacteria, and Gram-negative bacteria.

When using this reagent kit, the sample needs to be stained on two slides: one slide is stained with Calcofluor White M2R, a blue fluorescent dye that can be excited by UV and specifically binds to fungal cell walls. Another slide was stained with MycoLight Green JJ98 and Wheat Germ Agglutinin (WGA) - iFluor 594, JJ98 is a green fluorescent nucleic acid dye; WGA is a wheat germ agglutinin that can bind N-acetylglucosamine and N-acetylneuraminic acid residues. Due to the higher abundance and availability of N-acetylglucosamine in Gram positive bacteria compared to Gram negative bacteria, the fluorescent conjugate of WGA can serve as a probe for Gram positive bacteria. Therefore, the second slide can distinguish between Gram positive and negative bacteria. Gram positive bacteria can be simultaneously stained with red and green fluorescence, while Gram negative bacteria only show green fluorescence.

This reagent kit has the following characteristics:

1. The presence of microbial contamination in cultured cells can be determined by fluorescence staining within one hour.

2. It is possible to directly identify the approximate type of bacteria being infected, providing a basis for developing solutions in the next step.

3. The staining solution is in solution form and does not require dissolution. The working solution can be directly prepared.

4. Clear staining, bright fluorescence, high sensitivity, and more accurate results.

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Storage and transportation conditions

Store at -20 ℃ away from light, with a validity period of 12 months. Ice bag transportation.

Instructions for use

Preparation of reagents and working solutions:

1. BSA-physiological saline solutions: Dissolve 250mg BSA and 0.88g NaCl in 100ml distilled water, filter and sterilize, and prepare a BSA-physiological saline solution containing 0.25% BSA and 0.15M NaCl. (This reagent kit is not provided, please prepare it yourself)

2. WGA iFluor 594 working solutions: 2.5 μl WGA iFluor 594+47.5 μl BSA - physiological saline solution.

3. MycoLight Green JJ98 working solutions: 0.5 μl MycoLight Green JJ98+9.5 μl Distilled water.

4. Calcofluor White M2R working solutions: 0.5 μl Calcofluor White M2R+9.5 μl Distilled water.

Note: The working solutions can stabilize for several hours at room temperature.

Sample preparation:

1. Take 5ml of cell culture sample and transfer it to a 15ml sterile centrifuge tube. For adherent cells, only extract the supernatant of the culture medium; For suspended cells, it is necessary to collect the cells and culture medium together.

2. Centrifuge at 1000 g for 15 minutes.

3. Be careful to discard the supernatant and resuspend with 200 μl BSA- physiological saline solutions.

Note: You can first resuspend with 1ml BSA-physiological saline solution for washing, centrifuge and then resuspend with 200 μl BSA-physiological saline solutions, which can wash away nucleic acids and other components from the culture medium, reducing non-specific binding of dyes.

4. 20 μl sample was dropped onto two pre-cleaned glass slides with ethanol or methanol, labeled with "B" and "Y" respectively. "B" was used for bacterial detection, and "Y" was used for fungal detection.

Note: It is recommended to use PAP PAN or other immunohistochemistry pens to draw circles on the slide to limit the droplets.

5. Dry the glass slide with the sample added at 37 ℃ for about 10 minutes. Then, with the sample facing upwards, quickly pass through the flame three times and fix it.

Note: Be careful not to overheat. After each flame, feel the temperature on the back of your hand and control it to a temperature that your skin can tolerate.

6. 10 μl diluted Calcofluor White M2R working solutions was dropped onto the dry sample of slide Y and evenly cover it. Cover the cover glass carefully to avoid bubbles and store in dark.

Note: After covering the cover glass, the edge can be sealed with paraffin.

7. Add 50 μl BSA-physiological saline solutions onto the dry sample of slide B and cover it evenly. Let stand still for 5 minutes, be careful to inhale and discard liquids.

8. Add 50 µl diluted WGA iFluor 594 working solutions to the sample on slide B to evenly cover it. Let it stand for 5 minutes, be careful to aspirate and discard the liquid.

9. Add 10 µl diluted MycoLight Green JJ98 working solutions to the sample on slide B to evenly cover it. Be careful to cover the cover glass to avoid bubbles and store in dark.

Note: To reduce the green fluorescence background, the working solutions can be washed with BSA-physiological saline solutions first, and then covered with a cover glass. After covering the cover glass, the edges can be sealed with paraffin.

Fluorescence microscopy observation:

1. It is necessary to magnify at 400x or 1000x (using an oil mirror) for observation.
2. To observe fungal staining (slide Y), appropriate channels can be selected for blue fluorescence microscopy observation based on the excitation and emission wavelengths shown in the table below. Yeasts can be stained with bright blue fluorescence, appearing in a circular or oval shape, and often sprouting. Filamentous fungi can also exhibit filamentous blue fluorescence. In contrast, bacteria are hardly stained.
3. In order to observe the bacterial staining (slide B), first select the appropriate channel for green fluorescence microscopy observation based on the excitation and emission wavelengths in the table below. Gram-positive bacteria and Gram-negative bacteria will both present bright green fluorescence. Irregular green fluorescent staining may be dust or cell debris. Then, appropriate channels were selected based on the excitation and emission wavelengths in the table below for microscopic observation of red fluorescence. Gram-positive bacteria can exhibit bright red fluorescence, while Gram-negative bacteria are hardly stained.

Note: Fungi can also be stained with green or red fluorescence, but they are easily distinguished by their differences in morphology and size.

Fluorescent dyes	Calcofluor White M2R	MycoLight Green JJ98	WGA-iFluor 594
Ex（nm）	365	482	588
Em（nm）	435	512	604
Fluorescent color	Blue	Green	Red
Recommended filter	DAPI	FITC	Cy3/TRITC

Precautions:

1. The volume of liquid in the tube is small, please leave immediately before each suction to reduce liquid loss.
2. To maintain better detection results, WGA iFluor 594 recommends to be packaged and stored with 2.5 µl /tube, all dye working solutions are used and prepared as needed.
3. Fluorescent dyes all have quenching problems. It is recommended to avoid light during the detection process and complete the detection on the same day as much as possible after dyeing.
4. For your safety and health, please wear laboratory clothes and disposable gloves during operation.

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