

Cell Culture Contamination Detection Kit (C-7028)

Quick Facts

Storage upon receipt:

- -20°C
- Protect from light

Notes: Once reconstituted, avoid freeze-thaw cycles for Component C

Introduction

Molecular Probes' Cell Culture Contamination Detection Kit (C-7028) is a simple and effective procedure for screening tissue cultures for contamination by microorganisms. The kit not only serves to detect the contaminants, it also identifies the contaminant type. Three fluorescent dyes distinctly stain yeast (and other fungi), gram-positive and gram-negative bacteria in slide preparations. The kit provides results within one hour, and, by highlighting the microorganisms with different fluorescent colors, it helps a scientist choose an appropriate course of action.

In the application of this kit, a sample of the suspect culture is subjected to two slide-staining protocols. In one, the sample is stained with Calcofluor[®] White, a UV-excitable, blue fluorescent stain specific for fungal cell walls.¹⁻⁵ In the other, the sample is stained with SYTO[®] 9, a green fluorescent nucleic acid stain and also with Texas-Red[®]-X conjugated to wheat germ agglutinin (WGA). WGA is a lectin that binds to both *N*-acetylglucosamine and *N*-acetylneuraminic acid residues. Because the abundance and accessibility of *N*-acetylglucosamine is typically greater in gram-positive bacteria than in gram-negative bacteria, fluorescent conjugates of WGA serve as probes for gram-positive bacteria.⁶⁻⁸ Thus, the second treatment differentially stains gram-positive and gram-negative bacteria. The gram-positives are stained fluorescent red on the surface over a fluorescent green interior, while the gram-negatives are stained simply fluorescent green.

Materials

Kit Components

- SYTO[®] 9 green fluorescent nucleic acid stain (Component A), 100 µL in DMSO
- Calcofluor[™] White M2R blue fluorescent fungal cell wall stain (Component B), 100 µL in water
- Wheat germ agglutinin, Texas Red[®]-X red fluorescent gram positive stain (WGA-TRX, Component C), 1 mg
- Reconstituting buffer for Component C (Component D), 1 mL (0.1 M sodium bicarbonate, pH 8.3)

The kit provides sufficient material to perform approximately 200 contamination assays.

Storage and Handling

Upon receipt, this kit should be stored frozen at -20°C, upright and protected from light. Allow the reagents to warm to room temperature before opening the vials, and, before refreezing, seal all vials tightly. When stored properly, the components of this kit are stable for at least one year. Component C, the wheat germ agglutinin conjugate, is supplied as a lyophilized powder. Once reconstituted (see below), store the solution at 4°C, or, for longer storage, divide it into aliquots and freeze at -20°C. PROTECT FROM LIGHT, AND AVOID REPEATED FREEZING AND THAWING. The agglutinin in solution should be briefly centrifuged in a microcentrifuge before use; only the supernatant solution should be used in the experiment. This step eliminates protein aggregates that may have formed in solution and, thereby, reduces nonspecific background staining.

Caution: No data are available addressing the mutagenicity or toxicity of these reagents. Because Component A, the green-fluorescent cell stain, binds nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The stock solution of Component A should be handled with particular caution as the solvent is DMSO, which is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling this solution. As with all nucleic acid stains, solutions containing this reagent should be poured through activated charcoal before disposal, and the charcoal incinerated later to destroy the dye.

Experimental Protocol

Scientists at Molecular Probes have developed the following procedure and found it to be a simple and reliable method for identifying contaminants of tissue cultures.

Preparation

1.1 Reconstitute the lyophilized WGA-TRX (Component C) by adding 500 µL of reconstitution buffer (Component D) to the vial and then vortexing. For convenience and long-term stability, the solution can be stored at -20°C in 25 µL aliquots.

1.2 BSA-saline solution (0.25% bovine serum albumin in 0.15 M NaCl) is required, but is not provided in the kit. To prepare, dissolve 250 mg BSA and 0.88 g NaCl in 100 mL distilled water. The solution should be filtered to sterilize and to remove particulate matter.

Working Solutions

Dilute the stock solutions, as follows, to make working solutions sufficient for up to 10 assays. The working solutions are stable at room temperature for several hours, provided they are protected from light.

2.1 Dilute the reconstituted Component C (WGA-TRX): 25 µL + 475 µL of BSA-saline.

2.2 Dilute Component A, SYTO 9 solution in DMSO: 5 µL + 95 µL filtered distilled water.

2.3 Dilute Component B, Calcofluor White M2R solution in water: 5 µL + 95 µL filtered distilled water.

Sample Preparation

3.1 Take a 5 mL sample of the culture to be tested, and transfer it to a conical centrifuge tube. For cultures of adherent cells, sample only the covering medium; for suspension cultures, sample the medium and cells together.

3.2 Centrifuge the sample at 1000 × g for 15 minutes.

3.3 CAREFULLY pipet off and discard the supernatant. Resuspend the pellet in 200 µL BSA–saline. Note: Washing the cells in 1 mL of BSA–saline and recentrifuging may improve the subsequent staining reaction. Nucleic acids and other residual media components may bind the dyes in unpredictable ways resulting in low-level or variable cell staining or in high-background fluorescence.

3.4 Apply a 20 µL sample to two separate slides, one labeled “B” for the bacterial test, the other labeled “Y” for the yeast test. Pre-clean the slides with ethanol or methanol. It also helps to pre-mark the slides with circles to confine the droplets. For defining these “wells,” we recommend using either hot wax or a PAP PEN, available from The Binding Site, Inc. (San Diego, CA). Many standard marking pens are not suitable as color may leach into the solutions used. Allow both slides to dry at 37°C for about 10 minutes. Next, heat fix the samples by passing the slides, sample side up, over a weak flame. DO NOT OVER-HEAT. At no time should the slide become too hot to hold against the back of your hand. Repeat the heat treatment three times for each slide.

3.5 Pipet 10 µL of DILUTED Component B (Calcofluor White M2R) onto the fixed cells of Slide Y. Apply a coverslip, and seal with hot wax (or the equivalent). Set aside in darkness.

3.6 Pipet 50 µL of BSA–saline solution onto the fixed cells of Slide B. Be sure that the whole sample is covered. Let stand for 5 minutes. Remove this solution by pipetting it off, or simply shake it off.

3.7 Add 50 µL of DILUTED, reconstituted Component C (WGA-TRX) to Slide B. Again, confirm that the whole sample is covered. Let stand for another 5 minutes, and then remove the solution as before.

3.8 Add 10 µL of DILUTED Component A (SYTO 9) to Slide B. Apply a coverslip, and seal with hot wax (or the equivalent). To minimize the background of green fluorescence, you may wish to wash off the stain solution with 50 µL of BSA-saline before mounting the coverslip.

Fluorescence Microscopy

4.1 For observing the test for yeast and other fungi (Slide Y), use a filter set typically used for DAPI, Hoechst or aminomethylcoumarin applications. Under these conditions, yeast appear as bright blue fluorescent, spherical or ellipsoid cells, often budded. Filamentous fungi will also be stained bright blue fluorescent. Bacteria will be small in comparison and only slightly stained or unstained.

4.2 For observing the test for bacteria (Slide B), use first a fluorescein filter set. Any bacteria present, gram negative or gram positive will have bright green fluorescence. Yeast or other fungi present will also be green but easily distinguished by their characteristic shapes and larger size. Irregularly shaped, green fluorescence–stained material may be dust or cellular debris. Next, use a Texas Red or rhodamine filter set. Here, gram-positive bacteria will have bright red fluorescence, stained on their surface, while gram-negative bacteria will be barely visible, if at all. Yeast and other fungi will also have bright red fluorescence but, again, are easily recognized by their morphology.

References

1. J Cell Biol 123, 1821 (1993); 2. J Cell Biol 114, 111 (1991); 3. J Cell Biol 114, 101 (1991); 4. J Cell Biol 111, 143 (1990); 5. Biochim Biophys Acta 1035, 206 (1990); 6. J Clin Microbiol 10, 669 (1979); 7. Appl Environ Microbiol 56, 2245 (1990).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
C-7028	Cell Culture Contamination Detection Kit *200 assays*	1 kit

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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