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Technical Datasheet

EZdetect™ DAPI Stain Kit for *Mycoplasma* Detection

Product Code: CCK007

1. Introduction:

*Mycoplasma*s are small and simple parasitic prokaryotes that reside in the endosomes of mammalian cells and are widespread contaminants found in cell culture. They alone have been shown to alter the growth rate of cells in culture, induce chromosomal aberrations, influence amino acid and nucleic acid metabolism and cause membrane aberrations. Many *Mycoplasma* detection methods have been developed including microbiological cultivation on broth and agar, DNA staining using fluorophores such as DAPI or Hoechst derivatives, ELISA, immonofluorescence staining, PCR, biochemical detection etc. Each method has its own merits as well as demerits.

DNA fluorochrome staining is one of the convenient methods for *Mycoplasma* detection as it is rapid, sensitive and can detect both *Mycoplasma* and other prokaryotes in culture.

2. About the kit:

The EZdetect™ DAPI stain kit has been designed for detection of *Mycoplasma* based on rapid uptake of the fluorescent stain DAPI by cellular DNA. DAPI itself exhibits high permeability into cells; a property that facilitates quick uptake by the cells. It selectively binds minor grooves of the DNA. DNA-bound DAPI has approximately 20-fold greater fluorescence than non-DNA-bound DAPI. Its selectivity for DNA (over RNA) and high cell-permeability allows for efficient staining of the nuclei. The excitation maximum of dsDNA-bound DAPI is 358nm and emission maximum is 461nm. It can be excited either with a xenon mercury arc lamp or a UV laser and is detected through a blue filter.

Mycoplasma staining with DAPI appears as a fine particulate or filamentous staining over the cytoplasm at 100X magnification. Nuclei of the cells are also brightly stained by this method and thereby act as endogenous positive control for the staining procedure.

3. Kit Contents:

Contents		Kit Code	Storage
Code	**Description**	**CCK007-100**	
CCK007(A)	Hank's Balanced Salt Solution (10X)	1 x 10ml	Room Temperature
CCK007(B)	DAPI stain solution (10X)	1 x 10ml	2-8°C
CCK007(C)	Mounting Stain	1 x 10ml	2-8°C

4. Materials required but not provided in the kit:

- Sterile tissue culture grade water
- Carnoy's fixative (3:1 Methanol: Glacial acetic acid)
- Fluorescence microscope with barrier filter of 340-380nm and excitation filter of 460-500nm.
- Objective with appropriate magnification.
- Centrifuge tubes
- Glass slides
- Microscope coverslips

5. Directions for use:

Users are advised to review entire procedure before starting the assay

5.1 Preparation of reagents

5.1.1 Preparation of 1X Hank's Balanced Salt Solution:

Dilute the 10X Hank's Balanced Salt Solution (CCK007(A)) with sterile tissue culture grade distilled water in 1:9 proportion to obtain 1X Hank's Balanced Salt Solution.

5.1.2 Preparation of Working Solution of DAPI stain:
Dilute the DAPI stain solution (CCK007(B)) with 1X Hank's Balanced Salt Solution in 1:9 proportions.
*(**Note:** DAPI is a DNA-binding mutagen. Hence handle it carefully)*

5.1.3 Preparation of Carnoy's fixative:
Mix methanol and glacial acetic acid in 3:1 ratio.
*(**Note:** Prepare Carnoy's fixative freshly for every assay)*

5.2 Procedures for staining:
The kit has been designed to detect *Mycoplasma* in both suspension (non-adherent) and adherent cell cultures. Section 5.2.1 and 5.2.2 illustrate procedures for *Mycoplasma* detection in suspension and adherent cell cultures respectively.

5.2.1. Staining of suspension cells:
For detection of *Mycoplasma* in suspension culture, either of the two procedures mentioned below can be followed depending on user's choice and convenience.

Procedure I:
1. Aseptically aspirate the culture medium containing suspension cells from the culture vessel and transfer it to a sterile centrifuge tube.
2. Centrifuge the tube at 1000rpm for 10 minutes at room temperature.
3. Discard the supernatant and resuspend the pellet in 500µl of medium.
4. Add 1ml of freshly prepared Carnoy's fixative and mix well.
5. Centrifuge at 1000rpm for 10 minutes at room temperature.
6. Discard the supernatant, leaving behind 500µl of cell suspension.
7. Add 500µl of the working stain solution and mix well.
8. Allow it to stand for 15-20 minutes at room temperature, in dark.
9. Add one drop of the suspension on clean, grease-free slide and make a thin smear. Allow it to air dry.
10. Add 1 drop of mounting medium on the smear and put a coverslip on it.
11. Observe the slide under fluorescence microscope.

Procedure II:
1. Mix cells in the suspension thoroughly and evenly with pipette.
2. Aseptically place loopful of this suspension on a clean grease-free slide. Make thin smear and allow it to dry.
3. Add a few drops of Carnoy's fixative onto the slide, covering the smear completely.
4. Allow it to stand for 10 minutes at room temperature.
5. Decant the fixative off the slide.
6. Add 1ml of working stain solution, covering the entire smear.
7. Allow it to stand for 15-20 minutes at room temperature in dark.
8. Decant the stain solution and allow it to dry.
9. Add one drop of mounting medium on the slide and put coverslip on it.
10. Observe the slide under fluorescence microscope.

5.2.2. Staining of adherent cells:
Prerequisites for detection of *Mycoplasm*a in adherent cultures:
a) Cells should be grown.
b) The cells should be 50-80% confluent before use.
c) If the cells are grown in tissue culture flask as a monolayer, trypsinize the cells and follow the Procedure II given in section 5.2.1.

Procedure for staining of the cells grown on slides or chamber slides or on coverslips in Petri dish or 6-well tissue culture plates:
1. Aspirate the medium from culture vessel.
2. Add sufficient volume of freshly prepared Carnoy's fixative to cover the monolayer completely.
3. Allow it to stand for 10 minutes at room temperature.
4. Remove the fixative.
5. Add 1ml of working stain solution, covering the entire monolayer.
6. Allow it to stand for 15-20 minutes at room temperature in dark.
7. Remove the left over stain solution and allow it to dry.
8. Add small amount of mounting medium on the slide or coverslip.
9. Observe the slide under fluorescence microscope at 340/380nm excitation filter and 460/500nm emission filter.

6. Interpretation of the results:
6.1 Points to be considered before interpretation of the results:
a) The slides should be observed under a fluorescence microscope using objectives of magnification 40X or 100X. They can also be observed using a 100X oil immersion objectives.
b) To observe the specimens stained with DAPI, excitation and emission filters of wavelengths 340-380nm and 460-500nm, respectively, must be used.

6.2 Interpretation:

- If the culture is negative for *Mycoplasma* then it will show only nuclear fluorescence. Occasionally micronuclei or nuclear fragments from dead and disrupted cells will appear as spherical bodies. Their large size and brighter fluorescence will distinguish them from *Mycoplasma*.
- If the culture is positive for *Mycoplasma* then along with nuclear fluorescence it will also show extra-nuclear fluorescence. *Mycoplasma* can be identified by small pin point dots of fluorescence, either aggregated in clusters or scattered uniformly over the cytoplasm and sometimes in the intercellular spaces.

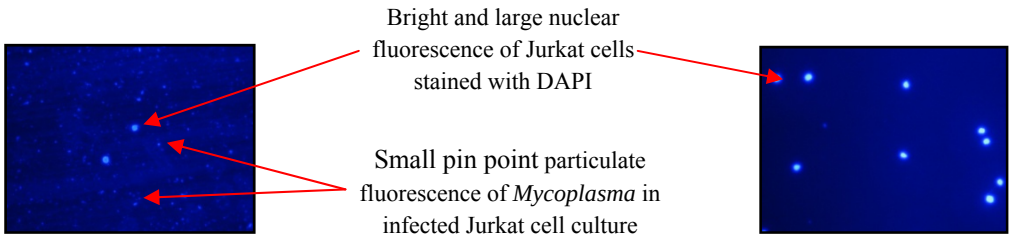

Fig. A: *Mycoplasma* infected cell culture

Fig. B: Non-infected cell culture

6.3 General guidelines for interpretation:

1. Bacteria, yeast and other prokaryotes show typical size, morphology and growth characteristics (i.e. chains, budding, mycelia, etc.).
2. Complete scanning of the specimen slide or test slide is necessary before interpreting the results because all the cells may not be infected with *Mycoplasma*. Incomplete scanning may result in false negative results.
3. If there is any doubt regarding the interpretation of the fluorescence, test should be repeated after generating a further subculture of the test cells in absence of antibiotics.
4. Further confirmation of the *Mycoplasma* infection can be done using other assays such as PCR, ELISA or direct growth on agar or in broth.

7. Storage and shelf life:

- DAPI stain is light sensitive. Store the stock solution and working solution at 2-8°C in dark. If not consumed frequently, the solutions can be aliquoted and stored at -20°C.
- Use before expiry date given on the label.

8. Advantages:

- **Time saving**: Detection of *Mycoplasma* in 45 minutes
- **Multipurpose**: Along with *Mycoplasma*, bacteria, yeasts, and other prokaryotes can also be detected
- **Uncomplicated detection**: *Mycoplasma* infection is detected as fine, particulate or filamentous staining over cytoplasm. This eliminates chances of false negative detection.
- **Sensitivity**: Because of high specificity of DAPI for DNA, *Mycoplasma* detection is very sensitive

9. Related products:

EZdetect™ Hoechst Stain Kit for *Mycoplasma* Detection
Code No: CCK008-100NO