Challenge

Determination of mycoplasma in cell culture and downstream biologicals

Solution

PCR and electrophoresis followed by simple analysis using automated bioimaging on UVP GelStudio

1-Click Imaging for Rapid Mycoplasma Analysis and Cell Culture Samples

Introduction

The detection of mycoplasma contaminants in cell culture and the downstream biologicals is critical due to the negative effects of mycoplasma on cellular physiology.

Mycoplasma contamination in continuous animal cell cultures has long been known to be a serious problem due to the wide range of mycoplasma induced effects on the cells in culture ranging from modifications to cellular gene expression to signal transduction pathway activation (1,2), leading to data that might be equally compromised due to the unintended effects of the Mycoplasma contamination.

The source of mycoplasma in biopharmaceuticals can be traced from the raw materials, cell lines and cell cultures used in manufacturing. Early detection therefore allows for fast decision making and corrective action to conserve downstream product integrity.

For commercial suppliers of cell lines, the ability to rapidly analyze cultures for microbial contamination, particularly contamination from Mycoplasma, is critical. A critical aspect is the monitoring of Mycoplasma contamination and the certification that there is no contamination in cell lines.

The new UVP GelStudio has several features that simplify Mycoplasma analysis. This includes automation scripts that permit out-of-the-box one click capture of a fluorescent PCR gel result. The automation extends annotating the gel image and saving the raw image for routine gel labeling and archiving.

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Materials and Methods

Sample Preparation

A 10 µl cell culture supernatant was boiled for 10 minutes and briefly centrifuged. PCR was run afterwards after 2 µl of the supernatant was added to the rehydrated PCR master reaction mixture. The PCR product was then analyzed using the bioimaging UVP GelStudio after running agarose gel electrophoresis.

Samples and Reagents

- The PCR mycoplasma test kit (PK-CA91-1024 from Promokine) was used to run PCR
- The kit contains primers, nucleotides, DNAs and hot start Taq polymerase needed for PCR
- Rehydration buffer
- Positive controls
- Agarose gel electrophoresis

Instrumentation

The UVP GelStudio uses customizable action buttons to automatically focus, capture, pseudo-color, and save images. Analysis also offers automated tools for identifying and quantifying electrophoretically separated DNA. Routine annotations can be saved and called up again for each gel, making labeling and documentation straightforward. In addition, the system has a lowlight (F1 .2) optical zoom lens allowing a wide variety of gel sizes to be analyzed, again the zoom setting can be preset for one-button automation. A wide variety of fluorescent dyes can be imaged, and the fluorescent emission filters can be easily exchanged to accommodate new dyes or multiplexing. There are also features that support 21 CFR part 11.

For experimentation there are full manual control of the camera, focus and zoom, exposure, and excitation and emission filters.

Results and Discussion

The UVP work studio provides an extremely fast and sensitive work studio for imaging and analyzing mycoplasma contamination. By means of agarose gel electrophoresis, human and mouse tumor cells that express GFP and RFP fluorescent proteins were analyzed for the presence of mycoplasma. A sample containing mycoplasma positive showed a distinct band at 265-278 bp whereas a non – mycoplasma present sample showed the internal control band and the negative control at 479 bp. LLC cells producing RFP fluorescent proteins and CT- 26 cells producing GFP fluorescent proteins tested positive for mycoplasma contamination. The presence of the internal control DNA as a distinct band on the gel revealed that the PCR run was successful and the data obtained is not erroneous (F1 .3).

The PCR mycoplasma kit obtained from Promokine (PK-CA91-1024) does not detect clinically important species such as *M. pneumonia, U. urelyticum* but identifies mycoplasma species such as *M. bovis* and *M. arthritidis*.

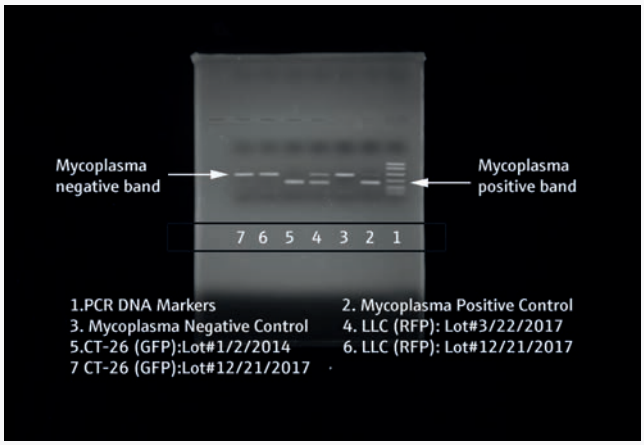

Figure 1.3: Agarose Gel of Mycoplasma PCR Test Results - Bioimaging results of agarose gel electrophoresis of PCR products from human and mouse tumor cells that express GFP and RFP fluorescent proteins. LLC (RFP) and C-26 (GFP) have the same number of base pairs as the Mycoplasma positive band DNA.

Conclusion

A robust method for detecting mycoplasma at very low concentrations in cell cultures has been developed. The UVP GelStudio with its innovative design perfectly fits in the laboratory workflow and is simple and accurate for the quantification and identification of mycoplasma in cell cultures. Fluorescence DNA on a gel can be imaged and the raw image labelled and analyzed. Data generated from bioimaging allows for straightforward documentation and also helps in archiving and referencing electrophoretic results in the future. The studio's capability will reduce data loss and facilitate data analysis.

References

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