INSTRUCTIONS **GM Biosciences**

Visual-PCR™ Mycoplasma Detection Kit*

Catalog No. GM7036 GM7048

2008.10

GM7036 **Visual-PCR™ Mycoplasma Detection Kit** **15 Tests** (1 set of component A, B, C and D)
GM7048 **Visual-PCR™ Mycoplasma Detection Kit** **75 Tests** (5 sets of component A, B, C and D)

Contents: Component A, B and C: to prepare complete-PCR-cocktail
Component D: synthesized 16s RNA gene of Mycoplasma (*M. orale*) for positive control

Storage: Product shipped on ice, store at -20 ºC upon arrival
Complete-PCR-cocktail should be stored at 4 ºC once prepared, do NOT freeze

Introduction

Visual-PCR™ Mycoplasma Detection Kit is a simple, quick and sensitive way to visually detect mycoplasma. It bases on our proprietary Visual-PCR technology. The technology makes PCR amplification to change color. For the very first time, PCR is capable of being visually monitored by naked eyes in visible light. DNA extraction is not required and crude sample is directly used as PCR template. Post-PCR gel electrophoresis is unnecessary. Benefited from the Visual-PCR technology, routine mycoplasma screening becomes feasible and practical.

Protocol

Important Notes:

80% of lab technicians are mycoplasma carriers and human-sourced species accounts for 50% mycoplasma contaminations. It is not uncommon that mycoplasma is widely spread in the whole laboratory. In order to prevent false positive results, it is strongly recommended to follow the guidelines as below.

Working area: Perform the following 1st and 2nd step in separate area. A PCR hood is strongly recommended for the 1st step.

Pipette: Always use pipettes which have never been used for cell related experiments. Pipettes, used for adding cell culture components (penicillin/streptomycin, growth factor, amino acid, FBS, culture medium, etc.) and stimulating reagents (chemical, cytokine, chemokine, etc.), washing cells, aliquoting cells, pipetting cells, are likely to be mycoplasma contaminated. Designate a 20ul-200ul pipette for preparing and aliquoting complete-PCR-cocktail, and a 10ul pipette for adding samples and positive control.

Pipette tip: Filter pipette tip is a must.

Lab mask: To block spreading of aerosol from cough and sneeze, wearing laboratory mask is suggested when setting up PCR reaction.

If above notes are not strictly followed, **Negative Control** may be contaminated and it may give false positive result.

1. **Prepare complete-PCR-cocktail by mixing component A, B and C.** Briefly spin tube A, B and C. Mix the whole tube of A (~192ul) and B (~176ul) first, and then add the mixture of A+B into tube C to give a complete-PCR-cocktail. Mix thoroughly and aliquot 24ul of complete-PCR-cocktail for each reaction. A desired complete-PCR-cocktail should be purple or violet, and it contains all the components for PCR reaction. Store the remaining complete-PCR-cocktail at 4ºC (stable for 1 month). **Do NOT freeze it.**

   Note: For consistent and desired color formation, it is not recommend to mix only partial tube of A, B and C.

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2. **Add 1ul samples into each reaction.** Duplicate reactions for each sample are recommended. A variety of samples can be added:

   Adherent cell cultures: directly take 1ul culture from culture plate without trypsinization of cells.

   Non-adherent cell cultures: directly take 1ul culture from culture flask without cells removal.

   Frozen cell lines in liquid nitrogen: thaw the frozen cells and take 1ul for testing. Alternatively pick a small piece frozen material (<1ul) for testing without thawing the cell lines.

   Culture medium or other supplements (DMEM, RPMI-1640, FCS, FBS, Penicillin/Streptomycin, Non-essential Amino Acid, 1xPBS, cell freezing solution, etc): take 1ul for testing. Trypsin/EDTA is **NOT** suitable for testing.

   Throat swab: dip the tip in swab to take trace amounts (<0.1ul), pipette the tip up and down several time into complete-PCR-cocktail for testing.

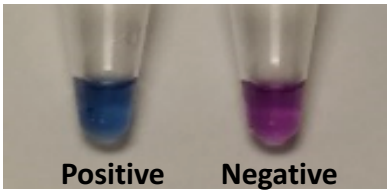
   Positive Control and Negative Control are necessary. Add 1ul component D into complete-PCR-cocktail as **Positive Control**. To rule out the possibility of cross-sample contamination when adding samples, it is suggested to close the tube of **Negative Control** all the time and add nothing for **Negative Control**.

   Note: Multiple freeze-thaw of component D for more than 2 times may fail the Positive Control. It is strongly recommended to aliquot component D into single-use tubes and stores them at -20 °C.

3. **Perform PCR:** 95 ºC 2 min, 60 cycles of (95 ºC 20 sec, 70 ºC 30 sec). PCR machine equipped with heated lid is necessary.

4. **Visually check the color of PCR results.** After PCR, chill the PCR tube on ice, and then check the color of reaction by naked eyes in visible light. **Positive Control** and mycoplasma-positive samples are blue or sky-blue. Sky-blue means high titer of mycoplasma contamination, whereas blue color means medium or low titer of mycoplasma. **Negative Control** and mycoplasma-negative samples§ are purple or violet. If the sample shows color between positive and negative, repeat the reaction to confirm the result.

   Note: Never open PCR tubes to prevent the PCR product aerosol from spreading and contaminating the whole laboratory. If Negative Control is blue unexpectedly, it indicates that Negative Control is contaminated by Mycoplasma. See "Important Notes" above to rule out the problem.



Troubleshooting and FAQ: ***http://www.gmbiosciences.com/products_Mycoplasma_FAQ.htm***

§ The detection limit of the kit is 10 copies/reaction, which is 100- 10,000 fold less than a typical mycoplasma contaminated culture (10^6-10^8 copies/ml).
* Patent pending

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