United States Department of Agriculture
Animal and Plant Health Inspection Service
Center for Veterinary Biologics
P. O. Box 844
Ames, IA 50010

1. Reagent Name: *Escherichia coli* harboring the pMyco plasmid (*E. coli*/pMyco)

2. Strain or Source: N/A

3. Lot Number: 091510B

4. Fill Date: September 17, 2010

5. Expiration Date: Not applicable, the stability of this reagent will be monitored by the Bacteriology Laboratory, Center for Veterinary Biologics.

Precautions: This reagent does not present a hazard to laboratory personnel if handled in a manner consistent with Biosafety Level-1 (BSL-1) recommendations.

6. Intended Use: This *E. coli* culture harbors a plasmid that serves as a positive control in the Polymerase Chain Reaction (PCR) detection method for testing biological agents and products for *Mycoplasma* contamination as described in **BBPRO1006**.

7. Instructions for Use: To expand as stock culture, thaw one vial of *E. coli*/pMyco culture on ice. Inoculate 50 mL of pre-warmed Luria Bertani broth (supplemented with 50 µg/mL kanamycin) with 1 mL thawed *E. coli*/pMyco culture. Incubate with shaking for 18-24 hours at 35°- 37°C. Add 5.0 mL sterile glycerol to the culture, mix thoroughly, and dispense aliquots into sterile cyrovials. Store the dispensed stock culture at -70°C or colder.

To use the *E. coli*/pMyco reagent as described in **BBPRO1006**, the pMyco plasmid must be purified from the *E. coli* host. Thaw one vial of stock *E. coli*/pMyco culture on ice. Isolate the pMyco plasmid DNA using a standard plasmid purification protocol or a commercial plasmid purification kit. Elute the plasmid DNA with sterile Tris-EDTA (TE) buffer (pH 8.0). Measure the DNA concentration of the pMyco plasmid preparation using a micro-spectrophotometer. Record the DNA concentration in ng/µL. Concentrated pMyco plasmid preparations may be stored at 3°- 7°C for up to 1 year or at -70°C or colder for longer storage.

The recommended use concentration of purified pMyco DNA is 0.5 ng/µL, however, the optimal concentration may be determined by titration using the PCR assay described in **BBPRO1006**. Make a working dilution of the pMyco plasmid preparation in sterile TE buffer. The working dilution of pMyco plasmid preparation may be stored at 3°- 7°C for up to 6 months.

8. Test of Reagent: Purity testing of the *E. coli*/pMyco culture was conducted by the method described in 9 CFR 113.27(d). Sequence confirmation of the plasmid was conducted by nucleic acid sequencing**.** The recommended use concentration of purified pMyco DNA was determined empirically using the PCR assay described in **BBPRO1006**.

9. Container Size, Type, Weight, or Volume: 1.5-mL screw-capped cryovial containing 1.0 mL *E. coli*/pMyco culture

10. Storage Conditions: Store at -70°C or colder.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6140 or FAX (515) 337-7673

12. Origin and Passage History: The *E. coli* host strain TOP10 and plasmid vector pCR®4-TOPO® were obtained from Invitrogen™.

13. Method of Preparation: *E. coli*/pMyco lot 091510B is a recombinant *E. coli* host strain harboring the plasmid pMyco. The pMyco plasmid was constructed by inserting a 462 base pair PCR amplicon generated from the 16S rDNA gene of *Mycoplasma hyorhinis* strain ATCC 17981 into the cloning vector pCR®4-TOPO®. An internal 86 bp segment was deleted from the PCR insert; the linear DNA was then circularized with T4 ligase and transformed into the *E. coli* host strain TOP10. The recombinant *E. coli* was expanded overnight in LB broth supplemented with kanamycin. Glycerol was added to the culture as a cryoprotectant prior to being aliquoted and frozen.

14. Other: The quality of a pMyco plasmid preparation can quickly deteriorate and is generally associated with unfavorable storage conditions, introduction of endonucleases from frequent handling, and/or low DNA concentration. If no amplicon is generated from the pMyco positive control, the following steps may be taken, 1) make a new pMyco plasmid prep; 2) perform a titration to determine the optimal DNA concentration of the pMyco positive control in the PCR reaction.

Reagent orders and feedback should be sent *including phone number* to the following email address: CVB@aphis.usda.gov

Reagent orders forms (APHIS 2018) are available from:
https://www.aphis.usda.gov/library/forms/pdf/APHIS_2018.pdf

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