

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 **CVB-PRO-0006**.
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 cryovials. Store the dispensed stock culture at -70°C or colder.

To use the *E. coli*/pMyco reagent as described in **CVB-PRO-0006**, 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 **CVB-PRO-0006**. 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 **CVB-PRO-0006**.
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) 337-6100.
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 464 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: VS.DB.CVB.Reagent.Requests@usda.gov

Reagent orders forms (APHIS Form 2018) can be found on the CVB website.