**Reagent Preparation Sheet.**

**Lysozyme Solution, pH 6.0, suspension solution**

**PCR Suspension Solution**

**Date prepared:**

**By:**

1. **Hazard Assessment**

To protect yourself from any possible hazards associated with this task wear eye protection. You should also wear latex, nitrile, or vinyl gloves and a lab coat with long sleeves. To protect your legs and feet wear closed shoes and long trowsers. Do not wear sandles, shorts or a short skirt. Wash your hands before eating and when leaving the laboratory. You should review the MSDS for any chemical used in this procedure. In case of a spill with a toxic chemical remove all contaminated clothing and wash affected areas with copious quantities of water. Check location of the nearest safety shower. Eyes should be washed copiously for 15 minutes.

1. **Reagent**

Mutanolysin and Lysozyme dilution for SeM PCR

1. **Purpose of Reagent**

Serves to break down insoluble cell walls to allow for exposure of soluble cellular elements in gram positive bacterial species such as *S. equi.*

1. **To Prepare a Working Dilution**

250 units/mL of Mutanolysin are needed and 2 x 10^6 units/mL of Lysozyme for every sample.

200 µL of solution are required per run.

200 µL x 41 samples = 8,200 µL = 8.2 mL

Combine 8.2mL of Gram-Positive Lysis Solution with 2,050 units of Mutanolysin and 164 x 10^5 units of Lysozyme.

Pipet the solution up and down to combine.

Aliquot into 200 µL aliquots for storage.

1. **Reference**

Sigma-Aldrich http://www.sigmaaldrich.com/technical-documents/protocols/biology/enzymatic-assay-of-mutanolysin.html