

**SOP: M031**

**Written: 1/13/23 DRM**

**Preparation of GAS + 20% Glycerol Stock Solution**

**Materials and Reagents:**

1. GAS Media
2. 80% glycerol stock solution
3. 16 x 100mm glass tubes with screw caps
4. 16 mini stir bars
5. P1000 pipetmen with tips
6. Tube rack
7. BioSafety Cabinet
8. Autoclave

**Protocol:**

1. \_\_\_\_\_ Prepare the 1L of GAS media (SOP:M001).
2. \_\_\_\_\_ Place glass tubes into tube rack and place a mini stir bar into each tube.
3. \_\_\_\_\_ Prepare the BioSafety cabinet (SOP:SP041).
4. \_\_\_\_\_ Aseptically place all needed materials and reagents into the BioSafety Cabinet.
5. \_\_\_\_\_ Using a Pipetman, add 1.6mL (1600uL) GAS media into each tube.
6. \_\_\_\_\_ Next, add 0.5mL (500uL) of the 80% glycerol stock solution to each tube.
7. \_\_\_\_\_ Loosely secure the cap onto each tube.
8. \_\_\_\_\_ Remove the tube rack from the BioSafety cabinet. Attach a strip of autoclave tape to the rack and autoclave using a liquid cycle (slow exhaust) at 121°C for 45 minutes.

**Reference:**

Takayama, K., H. K. Schnoes, E. L. Armstrong, and R. W. Boyle. 1975. Site of inhibitory action of isoniazid in the synthesis of mycolic acids in *Mycobacterium tuberculosis*. *J. Lipid Res.* 16: 308-317.