

**SOP: M014.2**  
**Updated 01/11/23 DRM**

**Preparation of SPAS medium protocol**

**Materials and Reagents:**

1. Milli-Q water
2. Beaker, 1 liter
3. Magnetic stir bar
4. Magnetic stir plate
5. BactoCasitone (BD Science BD225930)
6. Ferric ammonium citrate (Sigma F-5879)
7. Potassium phosphate, dibasic anhydrous (VWR MK709208) (note 1)
8. Citric acid, anhydrous (VWR JT0122-1)
9. L-Alanine (Sigma A-7627)
10. Magnesium chloride, hexahydrate (VWR MFCD00149781)
11. Potassium sulfate (VWR MK714004)
12. Ammonium chloride (VWR MK338412)
13. Sodium hydroxide, 10 M
14. Sodium pyruvate
15. Graduated cylinder, 1 liter
16. Autoclave

**Protocol:**

1. \_\_\_\_\_ Pour 1L of Milli-Q water into a 1 liter beaker.
2. \_\_\_\_\_ Add magnetic stir bar to beaker and place on magnetic stir plate.
3. \_\_\_\_\_ Add 0.3 g of BactoCasitone.
4. \_\_\_\_\_ Add 0.05 g of ferric ammonium citrate.
5. \_\_\_\_\_ Add 4.0 g of potassium phosphate, dibasic anhydrous.
6. \_\_\_\_\_ Add 2.0 g of citric acid.
7. \_\_\_\_\_ Add 1.0 g of L-alanine.
8. \_\_\_\_\_ Add 1.2 g of magnesium chloride.
9. \_\_\_\_\_ Add 0.6 g of potassium sulfate.
10. \_\_\_\_\_ Add 2.0 g of ammonium chloride.
11. \_\_\_\_\_ Make sure all components are completely in solution.
12. \_\_\_\_\_ Add 1.8 ml of 10 M sodium hydroxide.
13. \_\_\_\_\_ Make sure the sodium hydroxide is completely in solution.
14. \_\_\_\_\_ Add 4.4g of Sodium pyruvate.
15. \_\_\_\_\_ Make sure all reagents are fully dispersed.
16. \_\_\_\_\_ Measure the pH, and adjust to 6.6
17. \_\_\_\_\_ Autoclave on 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.