

## SASM Definition (1L SASM)

<i><b>Salts</b></i>	1L	10X	
KH <sub>2</sub> PO <sub>4</sub>	1 g	10 g	Use KCl and NaOH to adjust the pH to 7.0. Use 100mL of 10X Salt Stock for 1L of SASM
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	1 g	10 g	
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1 g	10 g	
NaCl	10 mg	100 mg	
<i><b>Carbon Source</b></i>	1L		
D-Glucose	10 g		20% stock solution can be prepared
<i><b>Vitamines</b></i>	1L	125X (500ml)	
Pyridoxine·HCl	1 mg	62.5 mg	Light shield the stock solution and keep it at 4 C.
Calcium Pantothenate (B <sub>5</sub> )	2 mg	125 mg	
Niacin (nicotinic acid)	2 mg	125 mg	Use 8ml of 125X vitamin stock solution to final 1L of SASM.
Riboflavin	1 mg	62.5 mg	
Folid acid	0.1 mg	6.25 mg	
Thiamine·HCl	2 mg	125 mg	
Biotin (B <sub>7</sub> )	0.1 mg	6.25 mg	
Inositol	1 mg	62.5 mg	
<i><b>Nucleosides</b></i>	1L		
Adenine	5 mg		Add directly. Stock solution cannot be prepared due to low solubility of nucleosides. Heating (autoclave) helps.
Uracil	5 mg		
Cytosine	5 mg		
Guanine	5 mg		
Xanthine	5 mg		
<i><b>Trace Metals</b></i>	1L	500X (100 ml)	
MgSO <sub>4</sub> 7H <sub>2</sub> O	200 mg	10 g	Trace metal stock solution (500X) can be prepared at low pH (~3.0). Prepare a separate FeSO <sub>4</sub> stock solution to prevent precipitation over time. Use 2ml of 500X stock to make 1L SASM.
MnSO <sub>4</sub> 7H <sub>2</sub> O	10 mg	0.5 g	
CuSO <sub>4</sub> 5H <sub>2</sub> O	1 mg	50 mg	
ZnSO <sub>4</sub> 4H <sub>2</sub> O	1 mg	50 mg	
FeSO <sub>4</sub> 5H <sub>2</sub> O	10 mg	0.5 g	
<i><b>Amino Acids</b></i>			

All L-amino acids	100 mg	<p>TYR has low solubility so no stock solution can be prepared. Dissolve 100 mg of TYR first.</p> <p>Prepare 10 g/l stock solution containing amino acids ARG, THR, SER, HIS, MET, and ASN.</p> <p>Prepare 5 g/l stock solution containing amino acids PRO, ILE, LEU, VAL, and PHE.</p> <p>Prepare 5 g/l stock solution containing amino acids ASP, CYS, and TRP.</p> <p>Add ALA, GLY, LYS, GLU, and GLN, separately depending on the isotope labeling scheme.</p>
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### **General description for preparing SASM and growing *S. aureus***

#### **Preparing 1L SASM**

1. To 500 ml of Grade I water add nucleosides (warming is needed to dissolve)
2. Add 100 mg of L-Tyr
3. Cool down the media to room temperature
4. Add 100 ml salt stock solution and 10 g of D-glucose
5. Add amino acids
6. Add 8 ml of vitamin stock solution
7. Adjust volume to 1L by adding water.
8. Add Trace Metals (drop by drop with stirring)
9. Adjust pH to 7.0
10. Sterile filter using 0.2  $\mu\text{m}$  pore size.
11. The media can be stored at room temperature or at 4C without light.

To selectively incorporate D-Ala into the peptidoglycan of *S. aureus* we add alanine racemase inhibitor alaphosphin to final concentration of 5  $\mu\text{g/ml}$ .

#### ***S. aureus* Growth**

1. Starter culture is prepared by picking several colonies of *S. aureus* from a bacteria lawn plate and transferred to two culture tubes each containing 6ml of Trypticase Soy Broth for overnight growth.
2. Add 5 ml of overnight culture to each two 1 L flasks each containing 500 ml SASM (1 % inoculum). The cells are grown 37 C and shaken at 200 rpm.
3. Monitor the growth and harvest the cells at OD 1.0 (at 660 nm).
4. Resuspend the pellet in ~20 ml of water and then sterilize
5. Wash the cells 2 times in water
6. Resuspend the pellet in ~20 ml of 40 mM triethanolamine (pH 7.0)
6. Freeze the bacterial suspension using liquid N<sub>2</sub> and then proceed with lyophilization.