

## **HS (Sueoka's high salt medium), HSA and YA**

from Sueoka, N. (1960) *Proc. Natl. Acad. Sci. USA* **46**, 83-91.

### **HS media (no acetate)**

5 ml Beijerinck's solution  
5 ml of phosphate solution  
1 ml of Hutner trace elements  
water to 1 L

### **HSA media (with acetate)**

5 ml Beijerinck's solution  
5 ml of phosphate solution  
1 ml of Hutner trace elements  
1.2 grams sodium acetate (anhydrous); for the hydrated salt use 2.0 g  
water to 1 L

### **YA media (with acetate and yeast extract)**

5 ml Beijerinck's solution  
5 ml of phosphate solution  
1 ml of Hutner trace elements  
1.2 grams sodium acetate (anhydrous); for the hydrated salt use 2.0 g  
4 grams yeast extract  
water to 1 L

#### 1) Beijerinck's solution (Complete salts solution)

NH <sub>4</sub> Cl	100 g
MgSO <sub>4</sub> · 7H <sub>2</sub> O	4.0 g
CaCl <sub>2</sub> · 2H <sub>2</sub> O	2.0 g

water to 1 liter

For sulfur-free salts solution, substitute 3.30 g MgCl<sub>2</sub> · 6H<sub>2</sub>O for the magnesium sulfate.

For N- medium for gametogenesis, omit the ammonium chloride.

#### 2) Phosphate solution

K <sub>2</sub> HPO <sub>4</sub> (anhydrous)	288.0 g
KH <sub>2</sub> PO <sub>4</sub>	144.0 g

water to 1 liter

#### 3) Hutner's trace elements

Keep the stock solutions at 4°C.

For plates or slants, use 12 grams agar per liter

## Hutner Trace Elements

[Hutner et al., Proc. Am. Philos. Soc. 94: 152-170 (1950)]

For 1 liter final mix, dissolve each compound in the volume of water indicated. The EDTA should be dissolved in boiling water, and the FeSO<sub>4</sub> should be prepared last to avoid oxidation.

EDTA disodium salt	50 g	250 ml H <sub>2</sub> O
ZnSO <sub>4</sub> · 7 H <sub>2</sub> O	22 g	100 ml
H <sub>3</sub> BO <sub>3</sub>	11.4 g	200 ml
MnCl <sub>2</sub> · 4 H <sub>2</sub> O	5.06 g	50 ml
CoCl <sub>2</sub> · 6 H <sub>2</sub> O	1.61 g	50 ml
CuSO <sub>4</sub> · 5 H <sub>2</sub> O	1.57 g	50 ml
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> · 4 H <sub>2</sub> O	1.10 g	50 ml
FeSO <sub>4</sub> · 7 H <sub>2</sub> O	4.99 g	50 ml

Mix all solutions except EDTA. Bring to boil, then add EDTA solution. The mixture should turn green. When everything is dissolved, cool to 70 degrees C.

Keeping temperature at 70, add 85 ml hot KOH (20%).

Cool to room temperature and bring to 1 liter final volume.

Usually the solution will be clear green initially but will turn dark red or purple over the next few days and leave a rust-brown precipitate. If no precipitate forms, or the solution remains green, check the pH. It should be about 6.7. If it's radically off this, try adding either KOH or HCl to adjust it.

The ritual calls for stoppering the flask with a cotton plug (to allow air exchange) and swirling it once a day for 1 to 2 weeks. Or better yet, use a 5 or 10 ml pipette (with a cotton filter at the top) pushed through a foam plug and bubble air through the solution for one week.

Filter through two layers of Whatman #1 filter paper; repeat if necessary until the solution is clear.

If the planets are correctly aligned and all the proper incantations have been done, the final product will approximate a good Bordeaux in color. However, even a subtle shift in pH can cause a color change. We had one batch that looked like grape Koolaid but worked fine.

Store refrigerated or frozen in convenient aliquots.

## Sulfur-free trace elements

Adapted from Hutner et al., Proc. Am. Philos. Soc. 94: 152-170 (1950)

For 1 liter final mix, dissolve each compound in the volume of water indicated. The EDTA should be dissolved in boiling water.

EDTA disodium salt	50 g	250 ml H <sub>2</sub> O
ZnCl <sub>2</sub>	10.0 g	100 ml
H <sub>3</sub> BO <sub>3</sub>	11.4 g	200 ml
MnCl <sub>2</sub> . 4 H <sub>2</sub> O	5.06 g	50 ml
CoCl <sub>2</sub> . 6 H <sub>2</sub> O	1.61 g	50 ml
CuCl <sub>2</sub> . 2 H <sub>2</sub> O	1.00 g	50 ml
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> . 4 H <sub>2</sub> O	1.10 g	50 ml
FeCl <sub>2</sub> .4 H <sub>2</sub> O	3.60 g	50 ml

As for the usual trace elements recipe, mix all solutions except EDTA. Bring to boil, and then add EDTA solution. The mixture should turn green. When everything is dissolved, cool to 70 degrees C. Keeping temperature at 70, add 85 ml hot KOH (20%).

Bring the final solution to 1 liter total volume and aerate for 1-2 weeks.

The color is the same as the original trace mix.