

**VERSION 3** 

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# OPEN ACCESS



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**Protocol status:** Working We use this protocol and it's

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### General Media Recipes: WA, PDA, LB, CMA, V8 V.3

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#### **ABSTRACT**

These are current general media recipes for (acidified) water agar, (acidified) potato dextrose agar, LB broth, cornmeal agar, and clarified V8 juice agar.

#### **GUIDELINES**

These are media recipes.

#### **BEFORE START INSTRUCTIONS**

#### Prepare the following:

- Turn on water bath if making more than 3-4 bottles of poured media, **or** if making  $P_5ARP/P_{10}ARP$ . Set to  $8^{\circ}$  50 °C.
- Prepare hood by spraying surfaces with ethanol and allowing hood to equalize for
   00:15:00 minutes.
- Open the necessary quantity of Petri plate bags and stack in hood (500 mL agar =~ 1.5 sleeves of 90 mm plates, 2 sleeves of 60 mm plates).
- Standardize pH meter, if applicable (aWA and aPDA).
- Thaw any antibiotics, if applicable.

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### **Making Media**

30m

1 Select media types from below:

### **Preparation**

STEP CASE

Water Agar (1.5%) 6 steps

WA and aWA (1.5%)

2 Water Agar preparation:

A	В	С
	500 mL	250 mL
Bottle size (mL)	1000	500
Agar (g)	7.5	3.75
RO Water (mL)	500	250

 $500 \text{ mL} \sim 1.5 \text{ sleeves of } 90 \text{ mm plates or } 2 \text{ sleeves of } 60 \text{ mm plates.}$ 

3 Add stir bar and stir contents well.



Place a loose orange cap. Label cap with autoclave tape (1.5% WA) and date.

4 Autoclave liquid setting for 20 minutes to sterilize.

## Acidified WA (aWA)

5 To make acidified WA (aWA):

Adjust to OPH 3.9 after autoclaving with [M] 10 % (V/V) lactic acid

■  $\Delta$  ~0.5 mL for 500 mL of media

## **After Pouring Media**

- 6 Mark plates with media color codes.
  - Turn off water bath.
  - Turn off autoclave, if no longer needed.
  - Spray inside of hood with ethanol.
  - Close filling port in pH meter, if necessary.
  - Tightly cap empty media bottles so agar doesn't dry out.
- When media has solidified, place in bags labeled with media name, your initials, and date.

Medium	Code
water agar	
acidulated w/lactic	add I after
acid	blue line
P <sub>5</sub> ARP	
P <sub>10</sub> ARP	ii
PDA	
½ PDA	II
¼ PDA	111
acidulated w/lactic	add I after
acid	black lines
Cornmeal	1
V8-juice + CaCO <sub>3</sub>	11
5/2 agar	
Malt agar	l II
Luria-Bertani agar	lii1
+ streptomycin	+
+ chloramphenicol	+
+ kanamycin	+
+ rifampicin	+
+ mefoxin	+
+ ampicillin	+

Bennett Lab media color codes.