

COMING SOON!



BioPaddles™ Colony Identification App

# NA/NA

Code 5550

Nutrient Agar (NA)

## USE:

Cultivation of a wide variety of non-fastidious bacteria.

Side 1 &amp; 2: Nutrient Agar (NA) (colorless, slightly hazy)



## APPLICATION

In the early 1900's, the American Public Health Association (APHA) suggested the formula of Nutrient Agar as a standard culture medium used in water testing.<sup>1</sup> Nutrient Agar continues to be a widely used general purpose medium for growing non-fastidious microorganisms. If required, enrichments can be added to this medium.

Nutrient Agar meets APHA and Association of Official Analytical Chemists (AOAC) standard methods.<sup>2 3</sup> Nutrient Agar is specified in Standard Methods for the Examination of Water and Wastewater procedures for the examination of food, dairy products, water, and other materials.<sup>4</sup>

## PADDLE AGAR

**Nutrient Agar (NA)** – The nitrogen, carbon, vitamins, and amino acids in Nutrient Agar are provided by enzymatic digest of gelatin and beef extract. Agar and a proprietary polymer are the solidifying agents.

Note: Good growth of nonfastidious organisms (bacteria) on Nutrient Agar will appear as translucent colonies.

## CULTURE CONTROLS

10-300 inoculum (CFU)

	NA Agar
<i>Bacillus subtilis</i>	GROWTH
<i>Escherichia coli</i>	GROWTH
<i>Aspergillus niger</i>	GROWTH
<i>Saccharomyces cerevesiae</i>	GROWTH

<sup>1</sup> American Public Health Association. 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, Washington, D.C.

<sup>2</sup> Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

<sup>3</sup> Marshall, R. T. (ed.). 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

<sup>4</sup> Vanderzant, C., and D. F. Splittstoesser (eds.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

**STORAGE / EXPIRATION**

Store tightly sealed BioPaddles™ in a cool, dry location (less than 68°F/20°C). Avoid temperature changes. BioPaddles™ may be refrigerated, but it is not necessary. Do not freeze. If freezing occurs, thaw (3-6 hours) under refrigeration temperatures (40°F; 4.4°C). Freezing can promote excess water loss and variation in media surface due to crystal formation. The average shelf-life is one year. Refer to expiration date (See: BBE stamped on vial). Discard if paddle agar appears oxidized (darkens from expected color). The expiration date applies to the medium in an intact container when stored as directed.

**SAMPLING**

**Liquids:** Twist to remove paddle from vial. Fill vial to 40 mL fill line with the liquid to be sampled. The 40 mL volume can be used to calculate Total Viable Count (TVC) and/or Total Colony Count (TCC). Replace paddle. Allow a contact time of 15 seconds. Remove the paddle. Empty the vial. Replace the paddle in the vial.



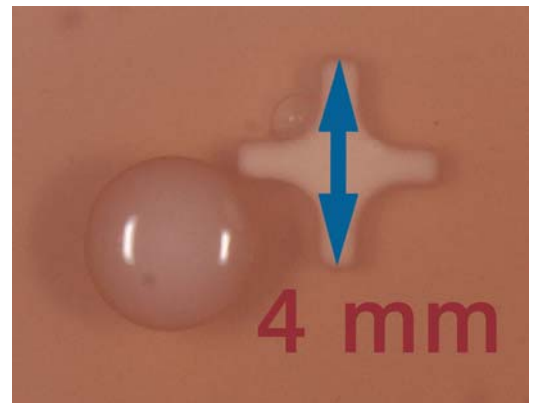
**Surfaces:** Twist to remove paddle from vial. Allow the paddle surface (10 cm<sup>2</sup>) to come into physical contact with the test surface. Recovery rate is about 50%. To insure an accurate recovery, gently sweep (or touch) the paddle to cover a 20 cm<sup>2</sup> area. Replace paddle in vial

**INCUBATION**


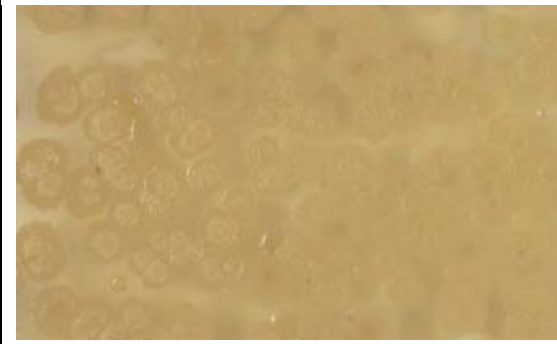


Temperature	Minimum Period	Optimal Period
35°C (bacteria)	72 hours	5-7 days
20-25°C (fungi)	5 days	7 days

**COLONY MEASURING**



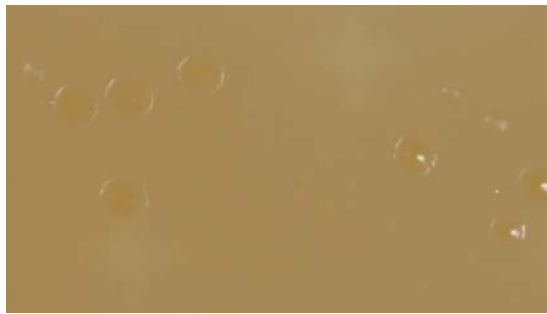
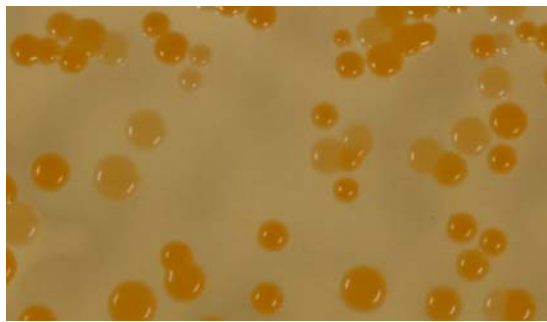
Each BioPaddles™ paddle has molded media attachment points that are 4mm in length (point-to-point). This feature provides a useful guidepost to estimating nearby colony size.



**IDENTIFICATION**

ORGANISM		NA		
ORGANISM	PHYSIOLOGY ◆ Precision Test Strip Available	GROWTH	COLONY	IMAGE
<i>Aspergillus niger</i>	<ul style="list-style-type: none"> <li>• Catalase (+)</li> <li>• Ascomycete</li> </ul>	+++	<ul style="list-style-type: none"> <li>• Granular</li> <li>• Jet black conidia w/ yellow/gray hyphae</li> </ul>	
<i>Bacillus spp.</i>	<ul style="list-style-type: none"> <li>• Lactose (-)</li> <li>• Indole (-) ◆</li> <li>• Oxidase (-) ◆</li> <li>• Catalase (+) ◆</li> <li>• Urease (-) ◆</li> <li>• Gram (+) Rod</li> <li>• 25 - 30°C</li> </ul>	+++	<ul style="list-style-type: none"> <li>• Translucent to dull, off-white; opaque</li> <li>• Smooth to rough</li> <li>• irregular / dendroid margins to spreading</li> <li>• 2-4mm</li> </ul>	
<i>Candida albicans</i>	<ul style="list-style-type: none"> <li>• Catalase (+)</li> <li>• Ascomycete</li> </ul>	+++	<ul style="list-style-type: none"> <li>• Cream</li> <li>• CVEG</li> <li>• 1-2mm</li> </ul>	
<i>E. coli</i>	<ul style="list-style-type: none"> <li>• Lactose (+)</li> <li>• Indole (+) ◆</li> <li>• Oxidase (-) ◆</li> <li>• Catalase (+) ◆</li> <li>• Urease (-) ◆</li> <li>• Gram (-) Rod</li> <li>• 35 - 37°C</li> </ul>	+++	<ul style="list-style-type: none"> <li>• Translucent; may be to dull, off-white, opaque</li> <li>• CVEG</li> <li>• 0.5 - 1.0mm</li> </ul>	

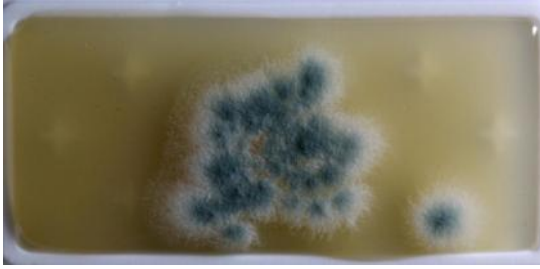
For *in vitro* diagnostic use only. This product should be used only by adequately trained personnel with knowledge of microbiological techniques in the laboratory.  
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ORGANISM		NA		
ORGANISM	PHYSIOLOGY ◆ Precision Test Strip Available	GROWTH	COLONY	IMAGE
<i>Enterobacter aerogenes</i>	<ul style="list-style-type: none"> <li>• Lactose (+)</li> <li>• Indole (-) ◆</li> <li>• Oxidase (-) ◆</li> <li>• Catalase (+) ◆</li> <li>• Urease (-) ◆</li> <li>• Gram (-) Rod</li> </ul>	+++	<ul style="list-style-type: none"> <li>• Yellow, translucent</li> <li>• CVEG</li> <li>• 1-2mm</li> </ul>	
<i>Pseudomonas fluorescens</i>	<ul style="list-style-type: none"> <li>• Lactose (-)</li> <li>• Indole (-) ◆</li> <li>• Oxidase (+) ◆</li> <li>• Catalase (+) ◆</li> <li>• Urease (-) ◆</li> <li>• Gram (-) Rod</li> <li>• Fluoresces blue-green under long-wave UV light (400-nm)</li> <li>• 25-30°C</li> </ul>	+++	<ul style="list-style-type: none"> <li>• Translucent to amber</li> <li>• Irregular; Spreading to confluent</li> <li>• Clear to grayish with dark centers (translucent edges)</li> <li>• Diffusible green-blue pigment</li> <li>• 2-4mm</li> </ul>	
<i>Lactobacillus delbrueckii</i>	<ul style="list-style-type: none"> <li>• Lactose (+)</li> <li>• Indole (-) ◆</li> <li>• Oxidase (+) ◆</li> <li>• Catalase (-) ◆</li> <li>• Urease (-) ◆</li> <li>• Gram (+) Rod</li> <li>• 40-44°C</li> </ul>	+++	<ul style="list-style-type: none"> <li>• Transparent / Gray</li> <li>• Rough; shiny</li> <li>• Convex, umbonate</li> <li>• 2 - 4mm</li> </ul>	
<i>Staphylococcus aureus</i>	<ul style="list-style-type: none"> <li>• Lactose (-)</li> <li>• Indole (-) ◆</li> <li>• Oxidase (-) ◆</li> <li>• Catalase (+) ◆</li> <li>• Urease (-) ◆</li> <li>• Gram (+) Sphere</li> <li>• 35 - 37°C</li> </ul>	+++	<ul style="list-style-type: none"> <li>• Yellowish-gold / Opaque</li> <li>• CVEG (butyrous)</li> <li>• 2 - 4mm</li> </ul>	

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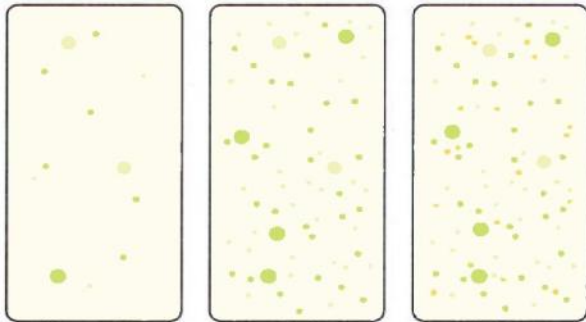
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ORGANISM		NA		
ORGANISM	PHYSIOLOGY ◆ Precision Test Strip Available	GROWTH	COLONY	IMAGE
<i>Penicillium chrysogenum</i>	<ul style="list-style-type: none"> <li>Catalase (+)</li> <li>Ascomycete</li> </ul>	+++	<ul style="list-style-type: none"> <li>Granular, velvet-like/powdery, flat</li> <li>Initially white, then various shades of green blue-green or yellow-green pigment</li> <li>3-5cm</li> </ul>	

**ENUMERATION**

**NA**

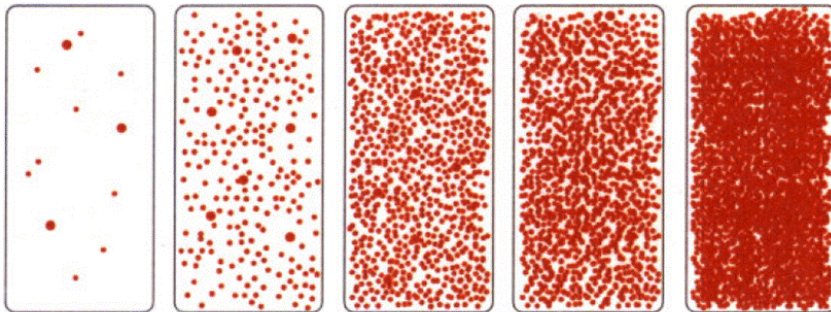


Very Light

Light

Moderate

**TVC/TCC**



100

1000

10000

100000

1000000

Approximate Colony Count per 100 mL

**TVC/TCC  
(Total Viable Count/  
Total Colony Counts)**

Colony Counts < 1000  
Count colonies  
TVC/TCC Count = Count x 2.5

Colony Counts > 1000  
Use chart  
TVC/TCC Count = Count x 2.5

(Based on a 40 mL sample)



Example:  
Inoculated NUT/TTC  
paddle showing  
approximately 1000  
CFU/100 mL.

### DISPOSAL

Twist to remove paddle from vial. Fill vial to 40 mL fill line with 1:9 dilution of household bleach (5.25% sodium hypochlorite). Replace paddle in vial. Allow 15 minute contact time. Remove paddle. Discard bleach solution. Replace paddle in vial and dispose. Alternatively, loosen cap and microwave for 30 seconds, autoclave, or incinerate.

### GLOSSARY:

<b>CVEG</b>	Convex, Entire, Glossy
<b>FED</b>	Full, Entire, Dull
<b>Catalase</b>	Cat (+) contains catalase enzymes that degrade cellular H <sub>2</sub> O <sub>2</sub> .
<b>Lactose</b>	Lac (+) bacteria can ferment available lactose in the medium producing an acid which lowers the pH. Lac (-) are non-fermenting.
<b>Indole</b>	Biochemical test to determine the ability of an organism to split indole from the amino acid tryptophan. <i>P. vulgaris</i> is indole (+) while <i>P. mirabilis</i> is indole (-).
<b>Oxidase</b>	OX (+) contains cytochrome c oxidase. In contact with an indicator turns dark blue if OX (+); colorless if OX(-).
<b>Urease</b>	UR (+) presence of enzyme urease which hydrolyzes urea into CO <sub>2</sub> & NH <sub>4</sub> .
<b>β-D-Glucoronidase</b>	Glu (+) Found in 97% of <i>E. coli</i> strains. The presence of <i>E. coli</i> is determined when both β-D-Glucoronidase and Indole are (+), and the organism is Gram (-).
<b>Gram</b>	Gram reaction