

# Instructions for Use

## EGG YOLK AGAR, MODIFIED

Cat. no. G215	Egg Yolk Agar, Modified, 15x100mm Plate, 18ml	10 plates/bag
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### INTENDED USE

Hardy Diagnostics Egg Yolk Agar, Modified is recommended for use as an enriched, non-selective and differential medium used in the cultivation, isolation and differentiation of *Clostridium* spp. and other anaerobic bacilli.

### SUMMARY

Hardy Diagnostics Egg Yolk Agar, Modified is based on the original formula for Egg Yolk Agar developed by McClung and Toabe for the isolation and differentiation of *Clostridium* spp. based on lecithinase and lipase production and proteolytic activity.<sup>(1)</sup> Microorganisms that possess the enzyme lecithinase break down lecithin to insoluble diglyceride and phosphorylcholine, which results in a white opaque zone of precipitation that spreads beyond the edge of the colony. Microorganisms that possess the enzyme lipase hydrolyze the free fats present in the medium to form glycerol and free fatty acids. Consequently, the release of insoluble free fatty acids results in the formation of an iridescent sheen (oil on water) that can be seen when the plate is held at an angle to a light source.<sup>(4,6)</sup> As compared to lecithinase, lipase is not diffusible and produces a reaction only on the surface of the agar in the immediate vicinity of the colony. Proteolysis is noted by the development of clear zones in the medium surrounding colony growth.

Hardy Diagnostics Egg Yolk Agar, Modified is a non-selective medium that contains enzymatic digests of casein and soybean meal that supply amino acids and other complex nitrogenous compounds to promote growth. Yeast extract provides B-complex vitamins required for growth. In addition, hemin and vitamin K<sub>1</sub> improve the growth of bacterial colonies. L-cystine is added as a reducing agent and is also an essential amino acid. Moreover, the media is enriched with an egg yolk suspension to detect the production of lecithinase and lipase and for the detection of proteolytic activity.

### FORMULA

Ingredients per liter of deionized water:\*

Pancreatic Digest of Casein	15.0gm
Vitamin K <sub>1</sub>	10.0gm
Sodium Chloride	5.0gm
Papaic Digest of Soybean Meal	5.0gm
Yeast Extract	5.0gm
L-Cystine	0.4gm
Hemin	5.0mg

Egg Yolk Emulsion	100.0ml
Agar	20.0gm

Final pH 7.0 +/- 0.3 at 25°C.

\* Adjusted and/or supplemented as required to meet performance criteria.

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

## PROCEDURE

### Specimen Collection:

As a general rule, infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

Specimens for anaerobic culture should be protected from air (oxygen) as much as possible during collection, transport, processing and incubation. Consult listed references for information on appropriate collection of anaerobic specimens.<sup>(2-6)</sup>

Use standard laboratory procedures to obtain isolated colonies from specimens.

For accurate results, this media should be reduced just prior to inoculation by placing it under anaerobic conditions for approximately 18-24 hours.

## Method of Use:

1. Prior to inoculation, allow medium to equilibrate to room temperature.
2. If the medium is not already pre-reduced, it must be reduced by placing it under anaerobic conditions for 18-24 hours.
3. Inoculate Egg Yolk Agar, Modified with a pure 24-72 hour culture. Streak the medium so as to obtain isolated colonies.
4. Immediately following inoculation, place the medium, in an inverted position (agar side up), in an anaerobic atmosphere and incubate at 35-37°C. for 48-72 hours.
5. Observe plates for the appearance of lecithinase and lipase production and proteolytic activity after 48 hours of incubation. Cultures should not be discarded as negative until after 7 days of incubation.

**Note:** If clostridia are suspected clinically and, upon gram stain, boxcar-shaped cells are observed, a direct Nagler test can be performed.<sup>(7)</sup>

## INTERPRETATION OF RESULTS

### Lecithinase:

A positive lecithinase test is noted by the appearance of a white, opaque, diffuse zone that extends into the medium surrounding the colonies.

A negative lecithinase test is indicated by the absence of a white, opaque zone extending from the edge of the colony.

### Lipase:

A positive lipase test is noted by the appearance of an iridescent sheen (oil on water) that can be seen when the plate is held at an angle to a light source.

A negative lipase test is indicated by the absence of an iridescent sheen.

### Proteolysis:

A positive test is indicated by clear zones in the medium surrounding colonial growth.

A negative test is indicated by the absence of a clear zone surrounding colonies within the medium.

## LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Hardy Diagnostics Egg Yolk Agar, Modified will not provide complete information for the identification of bacterial isolates. Additional biochemical and/or serological tests must be performed on colonies from pure culture for complete identification.

A negative lecithinase test should be compared to an uninoculated control plate, as lecithinase can diffuse throughout the entire agar plate and make interpretation difficult.

Some microorganisms may require up to one week to produce a positive lipase reaction.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture, media, Thioglycollate with Hemin and Vitamin K (Cat. no. K24) medium, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Clostridium perfringens</i> ATCC® 13124**	A	24hr	35°C	Anaerobic	Growth; lecithinase positive; white, opaque zone extending from edge of colonies, lipase negative; no sheen
<i>Clostridium sporogenes</i> ATCC® 11437	A	24hr	35°C	Anaerobic	Growth; lecithinase negative; lipase positive; iridescent sheen on agar surface when plate is held at an angle to the light source
<i>Bacteroides fragilis</i> ATCC® 25285**	B	24hr	35°C	Anaerobic	Growth; lecithinase and lipase negative; no reaction on agar

\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

## USER QUALITY CONTROL

\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

## REFERENCES

- McClung, L.S., and R. Toabe. 1947. The Egg Yolk Plate Reaction for the Presumptive Diagnosis of *Clostridium sporogenes* and Certain Species of the Gangrene and Botulism Groups. *J. Bacteriol.* Vol. 53; 139-147.
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[Ordering Information](#)

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