



# PRODUCT SPECIFICATION SHEET

## Bile Esculin Azide Agar, Modified (DM1322)

### Intended Use

Bile Esculin Azide Agar, Modified (DM1322) is recommended for selective isolation and presumptive identification of faecal *Streptococci*.

### Product Summary and Explanation

The value of esculin hydrolysis in the identification of enterococci was illustrated by Rochaix.<sup>(1)</sup> The enterococci were able to split esculin, but other streptococci could not. Meyer and Schonfeld incorporated bile into the esculin medium and showed that 61 of 62 enterococci were able to grow and split esculin, whereas the other streptococci could not.<sup>(2)</sup> Swan used an esculin medium containing 40% bile salts and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction.<sup>(3)</sup>

Bile Esculin Azide Agar was formulated by Isenberg et. al.<sup>(4)</sup> which is based on the original formula of Bile Esculin Agar formulated by Swan.<sup>(3)</sup> Isenberg et. al. modified Bile Esculin Azide Agar by reducing bile concentration from 40 to 10gm/l and added sodium azide.

### Principles of the Procedure

Bile Esculin Azide Agar, Modified is highly nutritious. Peptic digest of animal tissue and beef extract serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Sodium citrate acts as a buffering agent. Oxgall and sodium azide is used to inhibit most of the other accompanying bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies.<sup>(5)</sup> In slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc*, *Pediococcus*, *Lactococcus* species causing human infections give a positive bile esculin test.

### Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	17.00
Peptic digest of animal tissue	3.00
Yeast extract	5.00
Oxgall	10.00
Sodium chloride	5.00
Esculin	1.00
Ferric ammonium citrate	0.50
Sodium azide	0.25
Sodium citrate	1.00
Agar	13.50
Final pH : 7.1 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

### Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

### Directions

1. Suspend 56.25 grams of the medium in one liter of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

### Quality Control Specifications





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<b>Dehydrated Appearance</b>	Light yellow to brownish yellow homogeneous free flowing powder
<b>Prepared Medium</b>	Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates
<b>Reaction of 5.62% Solution</b>	pH : 7.1 ± 0.2 at 25°C
<b>Gel Strength</b>	Firm, comparable with 1.35% Agar gel

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for 24 - 48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
1.	<i>Enterococcus faecalis</i> ATCC 29212	50 -100	good-luxuriant	≥50%	positive reaction, blackening of medium around the colony
2.	<i>Staphylococcus aureus</i> ATCC 25923	50 -100	none-poor	≤10%	negative reaction
3.	<i>Streptococcus pyogenes</i> ATCC 19615	50 -100	none-poor	≤10%	negative reaction
4.	<i>Escherichia coli</i> ATCC 25922	≥10 <sup>3</sup>	inhibited	0%	--

The organisms listed are the minimum that should be used for quality control testing.

## Test Procedure

Refer to appropriate references for standard test procedures.

## Results

Refer to appropriate references and test procedures for interpretation of results.

## Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

## Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

## Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

## Packaging

**Product Name :** Bile Esculin Azide Agar, Modified

**Product Code :** DM1322

**Available Pack sizes :** 500gm

## References

1. Rochaix, 1924, Comt. Rend. Soc. Biol., 90:771.
2. Meyer and Schonfeld, 1926, Zentralbl. Bakteriol, Parasitenk. Infektionskr. Hyg. Abt. Orig. 99:402.
3. Swan, 1954, J. Clin. Pathol., 7:160.
4. Isenberg, Goldberg and Sampson, 1970, Appl. Microbiol. 20:433.
5. MacFaddin, 2000. Biochemical test for identification of medical bacteria, 3rd ed. Lippincott William & Wilkins, Baltimore, Md.

## Further Information

For further information please contact your local MICROMASTER Representative.



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