V1 _15/02/07

RAPID'*E. coli* 2 Agar

355-5299
356-4024

AREA OF APPLICATION

Selective chromogenic medium for direct enumeration of *Escherichia coli* and other coliforms in food products.

PRINCIPLE

The principle of the medium relies on simultaneous detection of 2 enzymatic activities : β-D-Glucuronidase (GLUC) and β-D-Galactosidase (GAL). The medium contains 2 chromogenic substrates. One substrate, specific to GAL, leads to blue coloration of colonies positive for this enzyme. The other substrate, specific to GLUC, leads to pink coloration of colonies positive for this enzyme.

Coliforms (GAL+/GLUC-) form blue to green colonies, *E. coli* (GAL+/GLUC+) form violet to pink colonies.

Detection of GLUC confers a high specificity to the culture medium. *Escherichia coli* is one of the only species of enterobacteria to possess this enzyme.

Note:
A few β-D-glucuronidase-negative strains of *E.coli*, e.g. *E. coli* O157, do exist. Some serovars of Salmonella and a few species of Shigella possess the enzyme β-D glucuronidase (< 1.5%).

AFNOR VALIDATION

The RAPID'*E.coli 2* technique (Enumeration of *E.coli* at 44°C) has AFNOR approval as a valid alternative method to the NF ISO 16649-2 standard for the enumeration of β-Glucuronidase-positive *Escherichia coli* at 44°C for all food products destined for human consumption, according to ISO 16140 protocol, under Attestation No: BRD 07/1-07/93.

The RAPID'*E.coli 2* technique (Enumeration of *E.coli* at 37°C) has AFNOR approval as a valid alternative method to the NF ISO 16649-2 standard for the enumeration of β-Glucuronidase-positive *Escherichia coli* at 44°C for all food products destined for human consumption, according to ISO 16140 protocol, under Attestation No : BRD 07/7 – 12/04.

The RAPID'*E.coli 2* technique (Enumeration of coliforms at 37°C) has AFNOR approval as a valid alternative method to the NF ISO 4832 standard for the enumeration of coliforms for all food products destined for human consumption, according to ISO 16140 protocol, under Attestation No: BRD 07/8 – 12/04.

AOAC VALIDATION

RAPID'*E.coli 2* agar method has been validated by the AOAC Research Institute under the Performance Tested Methods Status for selected foods (raw ground beef, raw boneless pork, fermented sausage, processed ham, processed turkey, frozen turkey breast, raw ground chicken, cottage cheese, processed ricotta cheese, raw milk, and dry infant formula). Cottage cheese and processed ricotta cheese were only validated at plate incubate temperature of 37°C. Plain yogurt, processed roast beef, chicken nuggets, raw chicken breast, vanilla ice cream, raw fish fillet and lettuce were initially examined. Results were not optimum so it has been excluded from the claim. This certification is for enumeration of coliform and *E. coli* bacteria only.

STANDARD REFERENCES

AOAC Official Method 966.24: Coliform Group and *Escherichia coli*.

NF ISO 16649-2: Food microbiology - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli.* Technique of colony count at 44°C by means of 5-bromo-4-chloro-3-indolyl- β-D-glucuronate acid.

NF ISO 4832: Microbiology - General guidelines for the enumeration of coliforms - Colony count method.

PRESENTATION

Ready-to-use
- 100ml x 6 bottles **355-5299**

Dehydrated
- 500g **356-4024**

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RAPID'*E. coli* 2 Agar

STORAGE / VALIDITY / BATCH

- Ready-to-use: +2 - 8 °C in a dark place.
- Dehydrated: +15-25°C, in tightly closed bottle in a cool dry place.
- Medium prepared by user: 1 month at +2 - 8°C in a dark place.
- Expiration date and batch number are indicated on the pack.

COMPLETE FORMULA

Meat peptones	5 g
Gelatin peptones	5 g
Sodium chloride	5 g
Yeast extract	3 g
Selective chromogenic mixture	6 g
Agar	13 g
Distilled water	1000 ml

Final pH (25°C) = 7.2 ± 0.2

OTHER PRODUCT(S) REQUIRED (NOT SUPPLIED)

Butterfield's Phosphate Buffer diluent

OTHER EQUIPMENT REQUIRED (NOT SUPPLIED)

- Autoclave
- Balance
- Blender
- Bottles, 125ml, autoclave-proof
- Hot plate, with stirring
- Sterile Petri dishes (Ø = 90 or 140 mm)
- Sterile pipettes (1 ml, etc.)
- Thermostatically controlled incubator capable of maintaining 37 ± 1°C and 44 ± 1°C
- Water bath accurate to ± 1 °C
- Weighing bags

PREPARATION OF THE DEHYDRATED MEDIUM

Always shake bottle before use
Dissolve 37g of powder in 1 liter of distilled water. Wait 5 minutes, and then mix until a homogenous suspension is obtained. Heat gently, agitating frequently, then bring to a boil until powder is completely dissolved. Autoclave at 121°C for 15 minutes.

500g powder reconstitutes 13.5L of medium

PROTOCOL

Preparation of sample
Weigh 50g of food sample and add 450ml of Butterfield's Phosphate Buffer diluent. Homogenize in a blender on high speed for 2 minutes.

Inoculation
Using a sterile pipette, transfer 1ml of sample (or 1ml of a 1:10 dilution of sample) into a sterile Petri dish. Rapidly pour 10-15ml of melted medium, cooled to 44-47°C. Swirl Petri dish to mix sample and agar. Leave to solidify on a cool, level surface.

Incubation
Turn the dishes over and incubate at:

- 37°C ± 1°C for 21 hours (± 3 h) for simultaneous enumeration of *E. coli* and coliforms.*
- 44°C ± 1°C for 21 hours (± 3 h) for enumeration of *E. coli*. *

* Incubation temperature for which the RAPID'*E.coli* 2 method has AFNOR validation (alternative method). In the context of AOAC-RI validation, 37°C and 44°C have been validated for simultaneous detection of coliforms and E. coli.

READING AND CONFIRMATION

Reading
- Coliforms (other than *E. coli*) form characteristic blue-green colonies.
- *E. coli* form characteristic pink to violet colonies.
- As *E.coli* is a species belonging to the coliform group, enumeration of total coliforms is achieved by adding the number of blue-green colonies and the number of pink-violet colonies.
- Only those plates that contain between 15-150 colonies should be retained.

Confirmation of characteristic colonies
- Confirm suspect isolated colonies according to classic confirmation test procedure described in the standard reference method.

PRECAUTIONS

- Respect Good Laboratory Practice. Appropriate protection, such as gloves and lab coat, should be worn when working with live organisms.

- Media that have come in contact with food samples should be considered contaminated and should be autoclaved prior to disposal.

- Avoid prolonged overheating and super cooling medium. The conserve optimal quality,

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the medium must not undergo more than 2 melt/solidify cycles.

- Molten agar must be cooled to 44-47°C before mixing with sample.

- The medium may present a frothy aspect after solidification in bottles. The quality of the medium is not affected and this disappears after melting.

- As development of colonies at the bottom of the Petri dish may interfere with reading, it is recommended that the time lapse between depositing the sample in the dish and dispensing the culture medium be limited to 1-2 minutes.

TECHNICAL SUPPORT

In the United States, for technical assistance please call (800) 4BIORAD. Select option 2 for technical support and option 2 again for the food science division. To place an order, please call (800) 4BIORAD and press option 1 for customer service.

QUALITY CONTROL

All products manufactured and marketed by Bio-Rad are incorporated into a quality assurance procedure from reception of raw materials through commercialization of the end product. Each batch of end product undergoes quality control, and is only marketed if it complies with acceptance criteria. Documentation concerning production and verification of each batch is archived. Material Safety Data Sheets are available on the web at www.foodscience.bio-rad.com

TEST QUALITY AND PERFORMANCE

STRAIN	Result after 24 hour	
	GROWTH	COLOR
Escherichia coli CIP 54.8	+	Violet
Klebsiella oxytoca SDP 12.1.1	+	Blue
Proteus mirabilis ATCC 25933	+	White
Staphylococcus aureus ATCC 25923	-	N/A