Harlequin® Chromogenic Agar for Salmonella Esterase (CASE) (NCM1006)

NCM1006	**500G, 5KG & 10KG DCM Packs**
NCM3008	90mm Pre-Poured Plates*

* Shipping restrictions may apply, enquire for regional variability.

Intended Use

CASE is a selective chromogenic agar for the detection of *Salmonella* that can be used as part of the ISO 6579-1:2017 workflow. It is not intended for use in the diagnosis of disease or other conditions in humans.

Description

CASE utilizes a dual chromogenic system to differentiate between *Salmonella* and non-target organisms that grow on the agar. The first chromogen is a target for esterase activity present in all *Salmonella* species, and results in blue/green colonies. The second chromogen is a target for ß-glucosidase activity, present in non-target organisms that are able to grow on the agar and also possess esterase activity. Utilization of the second chromogen masks the presentation of the first, resulting in black colonies in non-*Salmonella* organisms. All other organisms are either inhibited or grow colorless on the agar. The medium contains an opacity agent that provides a white background, facilitating easy reading and identification. The media is able to detect non-motile Salmonella (*S.* Pullorum and *S.* Gallinarum) as well as monophasic variants (*1*,*4*,[*5*],*12*:i:-). The media is also able to detect serovars that traditionally present weak esterase activity (*S.* Dublin), and lactose positive strains (*S.* Arizonae). According to ISO 6579-1:2017, subculture is performed separately from both Rappaport-Vassiliadis medium with Soya (RVS broth) and Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth.

Typical Formulation

Growth Mix	10.5 g/L
Selective Mix	11.0 g/L
Buffer	7.0 g/L
Opacity Agents	7.5 g/L
Chromogenic Mix	1.4 g/L
Agar	12.5 g/L

Final pH: 7.3 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 49.9 grams of medium in one liter of purified water.
2. Bring rapidly to the boil with frequent agitation and temper in a water bath to 45-50°C.

Preparation Precautions:

Selective agents are blended into the media meaning it does not require any supplementation. As such excessive heating during sterilization or prolonged holding may result in a loss of selectivity. Volumes up to 1 liter should be brought to the boil in no longer than 30 minutes, and should be held no longer than 1 hour after sterilization. Contact technical support further guidance on larger volume preparation.

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Please note the opacity agent requires proper boiling (i.e. visible bubbling) to present a smooth appearance in the plate. Failure to do this can result in a faint white precipitate. This is purely aesthetic and has no impact on performance.

Test Procedure

According to ISO 6579-1:2017, subculture is performed from both RVS (NCM0136) and MKTTn (NCM0126), by means of a 10µL loop streaked onto the surface of the agar. *Salmonella* should be confirmed by appropriate biochemical and serological techniques. Incubate at 37±1 °C for 18-24 hours.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and white/cream.

Prepared Appearance: Prepared medium is opaque and off-white. A very slight brown/black precipitate may be present at the base of the plates, this has no effect on performance.

Minimum QC:

Salmonella typhimurium ATCC 14028	Good Growth, Blue/Green Colonies
Salmonella enteritidis ATCC 13076	Good Growth, Blue/Green Colonies
Enterobacter aerogenes ATCC 13048	Growth, Black Colonies
Pseudomonas aeruginosa ATCC 27853	Inhibited

Results

Growth Characteristics		
Organism	**Growth**	**Color**
Salmonella spp.	Good Growth	Blue/Green
Enterobacter spp. *Klebsiella* spp	Growth	Black
Escherichia coli	Suppressed	Colorless
Shigella spp.	Suppressed	Colorless
Proteus spp.	Suppressed	Colorless to Brown

Expiration

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

Limitations of the Procedures

Some infrequent *Salmonella* isolates, typically avirulent environmental organisms that express ß-glucosidase activity may produce a black colony and thus a false negative. Whilst CASE either inhibits or masks any non-target organisms that produce esterase some isolates may result in a blue/green color, but with irregular morphology. All isolates can be confirmed by appropriate biochemical and serological methods.

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Storage

Store dehydrated culture media (NCM1006) at 2-8°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Store pre-poured plates (NCM3008) at 2-8°C away from direct sunlight

References

1. Cooke. V.M. et al, A Novel Chromogenic Ester Agar Medium for Detection of Salmonellae. *Appl. Environ. Microbiol*. Feb 1999 p. 807-812, Vol. 65, No. 2.
2. ISO 6579-1:2017 Microbiology of the food chain– Horizontal method for the detection, enumeration and serotyping of *Salmonella*. Part 1: Detection of *Salmonella* spp.

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