# Product : *Yersinia* CIN (CEFSULODIN-IRGASAN®-NOVOBIOCIN) AGAR BASE

## Also known as
CIN Agar; *Yersinia* Selective Agar

## Specification
Solid differential medium used for the selective isolation of *Yersinia spp.* from highly polluted samples, according to ISO 10273 standard.

## Formula * in g/L
| | | | |
|---|---|---|---|
| Special peptone | 20.000 | Magnesium sulfate | 0.010 |
| Yeast extract | 2.000 | Neutral red | 0.030 |
| Mannitol | 20.000 | Crystal violet | 0.001 |
| Sodium pyruvate | 2.000 | Agar | 15.000 |
| Sodium chloride | 1.000 | | |
| Sodium deoxycholate | 0.500 | | |

Final pH 7,4 ±0,2 at 25 ºC

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

## Directions
Suspend 30,25 g in 500 mL of distilled water and bring to the boil. Sterilize in the autoclave at 121ºC for 15 minutes. Let it cool to 50-55ºC and, aseptically, add the content of a vial of CIN Yersinia Selective Supplement (Art. No. DSHB3109). Homogenize and pour into plates.

## Description
Cefsulodin-Irgasan™-Novobiocin Agar CIN Agar was originally formulated by Schiemann (1979) for detection of *Yersinia enterocolitica*. He subsequently (1982) revised it by substituting sodium deoxycholate for bile salts and reducing the novobiocin content. It relies on the use of selective inhibitory components sodium deoxycholate, crystal violet, cefsulodin, Irgasan® and novobiocin. The basic principle involved is fermentation of mannitol with localised pH reduction which forms a red colony due to the neutral red and a zone of precipitation due to the deoxycholate.
The characteristic appearance of *Yersinia spp.* colonies after an incubation of 18-24 hours at 30ºC or 48 hours at 22ºC on CIN Agar in air, are round, pink, about 2 mm in diameter with a dark pink centre and surrounded with a precipitation zone. Confirmatory tests are required.
Typical colonies of *Yersinia enterocolítica* will develop as a red bull's-eye surrounded by a transparent border, but will vary considerably among serotypes in colony size, smoothness and the ratio of the border to centre diameter. Most other organisms that are capable of growing on this medium produce larger colonies (> 2 mm in diameter) with diffuse pinkish centres and opaque outer zones. Some strains of *Serratia*, *Citrobacter* and *Enterobacter* on CIN Agar may give a colonial morphology resembling *Yersinia enterocolitica*.
These organisms can be differentiated by simple biochemical tests.

**Necessary supplements**

Yersinia Selective Supplement (Art. No. DSHB3109))
Vial Contents:
Necessary amount for 500 mL of complete medium.
Cefsulodin 7,50 mg
Irgasan® 2,00 mg
Novobiocin 1,25 mg

Distilled water (Solvent)

## Technique
At present no single isolation procedure is available for the recovery of all pathogenic strains of Yersinia enterocolitica. The isolation procedure used will depend on the bio/serogroups of Yersinia spp. sought and on the type of sample to be examined. The ISO method for the detection of presumptive pathogenic Yersinia enterocolitica includes the parallel use of two isolation procedures:

1. Enrichment in Peptone, Sorbitol and Sels biliaire (PSB) Broth for 2-3 days à 22-25ºC with agitation or 5 days without agitation; plating on CIN Agar directly and after alkaline treatment and incubation for 24 hours à 30ºC.

2. Enrichment in ITC (Irgasan®-Ticarcillin-Chlorate) Broth for 2 days à 24ºC; plating on SSDC (Salmonella-Shigella-Deoxycholate-Calcium Chloride) Agar and incubation for 2 days à 30ºC.

## Quality control

**Incubation temperature:** 30 ± 2 °C **Incubation time:** 21±3h

**Inoculum:** Practical range 50- 100 CFU (Productivity) /$10^4$-$10^6$ CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018

| Microorganism | Growth | Remarks |
|---|---|---|
| *Yersinia enterocolitica* ATCC® 9610 | Good | - |
| *Escherichia coli* ATCC® 25922 | Partial inhibition | - |
| *Staphylococcus aureus* ATCC® 25923 | inhibited | - |

## References

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. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
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· WEAGANT, S.D. & P. FENG (2001) Yersinia, in "Compendium of Methods for the Microbiological Examination of Foods". 4th ed. Downes & Ito (Eds.) APHA. Washington. DC. USA.

## Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).