

FERRIC AMMONIUM CITRATE SUPPLEMENT

FACS-00P-010

- **Principle**

Ferric Ammonium Citrate Supplement is a sterile additive used in selective culture media for the detection of esculin hydrolysis, a characteristic reaction of *Listeria* spp. It is intended to be added to *Listeria* enrichment broth base for the preparation of Fraser broth (FRLB-001) or half Fraser broth (FRLB-0H1), where it contributes to the visual detection of presumptive *Listeria* growth.

The principle of the medium is based on the ability of *Listeria* spp. to hydrolyse esculin. The hydrolysis products react with ferric ions supplied by ferric ammonium citrate, producing a characteristic darkening or blackening of the medium. Therefore, development of a dark colour in the complete medium can be considered indicative of presumptive *Listeria* growth.

Selectivity is achieved through the combination of the complete medium components and the additional selective agents. Lithium chloride suppresses the growth of enterococci, which may also hydrolyse esculin, while nalidixic acid and acriflavine hydrochloride inhibit accompanying flora. This selective system facilitates the enrichment and presumptive detection of *Listeria* spp. before confirmation by appropriate microbiological and biochemical tests.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 11290-1.

- **Composition**

Ingredients	g/vial
Ferric Ammonium Citrate	0,250

- **Preparation**

Collect and prepare the samples, including any required dilutions and test volumes, in accordance with the applicable specifications, official standards, regulatory requirements, and expected analytical results.

Under aseptic conditions, reconstitute the vial with 6 ml of sterile diluent and mix until completely dissolved. Add the reconstituted supplement to 500 ml of sterilized *Listeria* Enrichment Broth Base (BLEB-IEP), previously cooled to approximately 50 °C. Add the corresponding selective agent to inhibit the growth of accompanying flora, then mix thoroughly.

Dispense the complete medium into sterile tubes and inoculate with the prepared samples. Incubate under aerobic conditions at 35 ± 2 °C for 24–48 hours. Depending on the sample type

or the requirements of the applicable method, alternative incubation temperatures or longer incubation periods may be necessary.

After incubation, subculture presumptive positive cultures or tubes showing characteristic darkening by streaking onto an appropriate recommended secondary culture medium for confirmation of *Listeria* isolation. The presumptive presence of *Listeria* spp. must subsequently be confirmed by appropriate microbiological and biochemical tests.

- **Application and use**

Recommended to use as a complement of the test of esculin hydrolysis.

- **Quality control**

Colour	Yellowish-brown
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- **Microbiological control**

Perform the analytical procedure in accordance with ISO 11133:2014/A1:2018; A2:2020.

Incubate under aerobic conditions at 37 ± 1 °C and evaluate the results after $24/44 \pm 4$ h.

Microorganism	ATCC	WDCM	Growth	Esculin Reaction
<i>L. monocytogenes</i>	13932	00021	Good	Positive (Black medium)
<i>Listeria monocytogenes</i>	35152	-	Good	Positive (Black medium)

- **Storage and shelf life**

Shelf life: 49 months. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

- **Sterility control**

Sterility is verified by inoculating 5 ml of the sample into 100 ml of TSB and 100 ml of Thioglycollate. The inoculated media are incubated for 48 hours at 30–35 °C and 48 hours at 20–25 °C. No microbial growth shall be observed after the specified incubation periods. Check at 7 days after incubation in same conditions.

- **Bibliography**

ATLAS, R.M. (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Florida.

FRASER, J.A. & W.H. SPERBER (1988) Rapid detection of *Listeria* spp. In food and environmental samples by esculin hydrolysis. J. Food Prot. 51:762-765.

ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

ISO 11290-1:2017 Standard. Microbiology of the food chain. Horizontal method for the detection and enumeration of *Listeria monocytogenes* and for *Listeria* spp.- Part 1: Detection Method

ISO 11290-2:2017 Standard. Microbiology of the food chain. Horizontal method for the detection and enumeration of *Listeria monocytogenes* and for *Listeria* spp.- Part 2: Enumeration Method

McCLAIN, D. & W.H. LEE (1988) Development of a USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. J.AOAC 71:660-664.

VANDERZANT, C & D.F. SPLITTSTOESSER (1992) Compendium of methods for the microbiological examination of foods. APHA. Washington. DC.

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.