

ECC Agar

E. coli & Coliform Chromogenic Medium | Catalogue No. AS-1438

1. Product Overview

ECC Agar (AS-1438) is a selective and differential chromogenic culture medium for the simultaneous detection and enumeration of *Escherichia coli* and total coliform bacteria from a single plate, without the need for confirmatory testing. Compliant with ISO 9308-1:2014, it is used across water quality testing, food microbiology, environmental monitoring, and pharmaceutical QC laboratories.

2. Mode of Action

The medium incorporates two chromogenic enzyme substrates. A β -D-galactosidase substrate is cleaved by the enzyme common to all coliforms, releasing a salmon-red chromophore. A β -D-glucuronidase substrate is cleaved specifically by *E. coli*, releasing a blue-indigo chromophore that combines with the salmon component to produce a distinct blue-violet colony colour. Tergitol 7 selectively suppresses Gram-positive background flora.

3. Product Specifications

Synonyms	ECC Agar; <i>E. coli</i> Coliform Detection Medium; Chromogenic Coliform Agar (CCA)
Medium Type	Selective, differential, chromogenic — dual enzyme substrate system
Standards	ISO 9308-1:2014 ISO 9308-2:2012 ISO 7218:2007 AS/NZS 4276 series ISO 11133:2014
Final pH (25°C)	7.0 ± 0.2
Sterilisation	DO NOT AUTOCLAVE — heat to boiling to dissolve, then cool to 45–50°C
Appearance (powder)	Beige to light tan, free-flowing, homogeneous powder
Appearance (prepared)	Light amber to pale yellow, slightly opalescent gel
Incubation	36 ± 2°C for 21 ± 3 hours, aerobically (ISO 9308-1:2014)
Storage (powder)	2–25°C, dry, tightly sealed, protected from light
Storage (prepared plates)	2–8°C; use within 2 weeks; protect from light
HS Tariff Code	3821.00.00

4. Formula — per litre

Ingredient	g/L	Role
Peptone (enzymatic digest of casein)	~15.0	Primary nitrogen source — amino acids, peptides, growth factors
Yeast extract	~3.0	B-vitamins, trace elements, growth co-factors
Sodium pyruvate	~1.0	Energy source; supports recovery of stressed/injured coliforms
Sorbitol	~1.0	Carbohydrate; supports coliform growth and recovery
Sodium chloride	5.0	Osmotic balance
Disodium hydrogen phosphate (Na_2HPO_4)	~2.7	pH buffer component
Sodium dihydrogen phosphate (NaH_2PO_4)	~2.2	pH buffer component
Tergitol 7 (sodium heptadecyl sulphate)	0.15	Primary selective agent — suppresses Gram-positive organisms
β -D-galactosidase chromogenic substrate	Chromogenic	Cleaved by β -D-galactosidase — salmon-red in all coliforms
β -D-glucuronidase chromogenic substrate	Chromogenic	Cleaved by β -D-glucuronidase — blue-indigo in <i>E. coli</i>
IPTG (isopropyl- β -D-thiogalactopyranoside)	0.1	Inducer — enhances β -D-galactosidase expression
Agar	~12–15	Solidifying agent

Total dissolution: ~36–42 g/L | Final pH 7.0 ± 0.2 at 25°C | DO NOT AUTOCLAVE

5. Preparation Protocol

- Suspend the required amount of dehydrated ECC Agar in purified or distilled water per the label dissolution rate (g/L). Mix well.
- Heat with frequent agitation to boiling. Continue for approximately 1 minute to dissolve completely. Avoid prolonged boiling. DO NOT AUTOCLAVE.
- Cool to $45\text{--}50^\circ\text{C}$ in a water bath. Do not allow the medium to set — pour while still molten.
- Pour 10–15 mL per 90 mm Petri dish and allow to solidify on a level surface.
- Dry the agar surface (lids ajar, room temperature, 30–60 minutes, or 37°C for 30 minutes) to prevent colony spreading.
- For membrane filtration: place the membrane filter grid-side up on the agar surface. Incubate at $36 \pm 2^\circ\text{C}$ for 21 ± 3 hours.

6. Colony Interpretation

Colony Colour	Organism / Group	Enzyme Activity
Dark blue to violet	<i>E. coli</i>	β -D-galactosidase + β -D-glucuronidase positive
Salmon-pink to red	Total coliforms (non- <i>E. coli</i>)	β -D-galactosidase positive only

Colony Colour	Organism / Group	Enzyme Activity
Colourless / no growth	Non-coliforms	Neither enzyme present — suppressed by Tergitol 7

E. coli count = blue-violet colonies. Total coliform count = blue-violet + salmon-pink/red colonies. Report as CFU per 100 mL of sample filtered (ISO 9308-1:2014). Note: some *Aeromonas* spp. may give false-positive blue-violet colonies — confirm with an oxidase test where relevant.

7. Quality Control — Organisms & CFU Specification

Organism (ATCC)	Inoculum (CFU)	Expected Result
<i>Escherichia coli</i> ATCC 25922	≤100 CFU	Good growth; dark blue to violet colonies
<i>Escherichia coli</i> ATCC 8739	≤100 CFU	Good growth; dark blue to violet colonies
<i>Klebsiella (Enterobacter) aerogenes</i> ATCC 13048	≤100 CFU	Good growth; salmon-pink to red colonies
<i>Citrobacter freundii</i> ATCC 43864	≤100 CFU	Good growth; salmon-pink to red colonies
<i>Enterococcus faecalis</i> ATCC 19433	≤100 CFU	Inhibited / partial growth; no typical colour
<i>Staphylococcus aureus</i> ATCC 25923	≤100 CFU	Inhibited / no growth; no typical colour

8. Applications

- Drinking water — ISO 9308-1:2014 membrane filtration, 100 mL sample volume
- Recreational water — bathing water, pools, beach water compliance
- Environmental water — rivers, lakes, stormwater, irrigation water
- Food and dairy — process water and hygiene monitoring
- Pharmaceutical QC — USP/EP process water testing for coliforms
- Environmental monitoring — surface sampling, wastewater and effluent compliance

9. Storage & Shelf Life

Dehydrated powder	24 months from date of manufacture when stored at 2–25°C, sealed, protected from light and moisture
Prepared plates	2 weeks at 2–8°C, protected from light

10. Literature & References

1. ISO 9308-1:2014. Water quality — Enumeration of *Escherichia coli* and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora. Geneva: International Organization for Standardization.
2. Farnleitner AH, et al. Performance validation of chromogenic coliform agar for the enumeration of *E. coli* and coliform bacteria in water. *Journal of Applied Microbiology*, 2004.
3. Manafi M, Kneifel W. A combined chromogenic–fluorogenic medium for the simultaneous detection of total coliforms and *E. coli* in water. *Zentralblatt für Hygiene und Umweltmedizin*, 1990;189:225–234.
4. ISO 7218:2007. Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations. Geneva: ISO.
5. ISO 11133:2014. Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media. Geneva: ISO.

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