



Escherichia coli (Migula) Castellani and Chalmers

25252™

Description

Strain designation: Chi 99

Deposited As: *Escherichia coli* (Migula) Castellani and Chalmers

Type strain: No

Storage Conditions

Product format: Freeze-dried

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always

used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:

ATCC Medium 3: Nutrient agar or nutrient broth

Temperature: 37°C

Handling Procedures

1. Open vial according to enclosed instructions.
 - 2. Using a single tube of #3 broth (5 to 6 ml), withdraw approximately 0.5 to 1.0 ml with a Pasteur or 1.0 ml pipette. Rehydrate the entire pellet.**
 - 3. Aseptically transfer this aliquot back into the broth tube. Mix well.**
 - 4. Use several drops of the suspension to inoculate a #3 agar slant and/or plate.**
 5. Incubate the tubes and plate at 37°C for 24 hours.
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Notes

Colonies on #3 agar are entire, glistening, circular, smooth, low convex, and translucent.

This strain requires vitamin B₁, leucine, and threonine for growth. The following is a method for checking one or more of the requirements:

1. Remove a small amount of growth from the 24 hour plate and suspend it in a tube of minimal medium.

2. Transfer a drop of this suspension to another tube of a minimal medium to dilute the suspension. Tube should show very light turbidity. As an alternate method, you may wish to centrifuge the suspension and wash several times with buffer or minimal medium; then dilute.

3. Prepare a series of minimal medium broth tubes with the various requirements added; one tube containing all, the rest with one each omitted.

4. Inoculate each tube with one drop of the cell suspension, and one tube of Nutrient broth as a control.

5. Incubate and check for growth for several days. The tube of Nutrient Broth and the tube with all the requirements added should be the only ones showing growth. If any of the others grow, this indicates the strain has lost the requirement for the substance that was omitted from that tube.

To avoid mutation or loss of specific requirements, avoid repeated transfers. These requirements are very easily lost.

The following is the formulation for a suggested minimal medium:

M9 Minimal Medium:

M9 Salts (X10) (*see below*), 100 ml

20% Glucose, 20 ml

0.1M MgSO₄, 10 ml

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Product Sheet

0.01M CaCl₂, 10 ml

Sterile water to 1000 ml

Autoclave each solution separately (15 lb for 15 min). Mix all components aseptically just before use.

M9 Salts (X 10 concentrate):

Na₂HPO₄ anhydrous, 60 g

KH₂PO₄ anhydrous, 30 g

NaCl, 5 g

NH₄Cl, 10 g

Water to 1000 ml

Dissolve in order indicated. Dispense into 100 ml bottles. Autoclave at 15 lb for 15 min.

For ATCC 25252, add the following for final concentrations of:

Vitamin B₁, 0.1 ug/ml

Leucine, 0.1 mg/ml

Threonine, 0.1 mg/ml

It may be desirable to maintain this strain on a medium containing streptomycin to maintain the resistance.

Additional information on this culture is available on the ATCC web site at www.atcc.org.

Material Citation

If use of this material results in a scientific publication, please cite the material in the

following manner: *Escherichia coli* (Migula) Castellani and Chalmers (ATCC 25252)

References

References and other information relating to this material are available at www.atcc.org.

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