LABORATORY FOR HEALTH, OMICS AND PATHWAY EXPLORATION

Emulsification Index (E24) Assay

A defined cell-free assay for assessing 24-hour emulsion stability in approved, non-viable sample material.

DOCUMENT	VERSION	ISSUED
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Safety boundary: The procedure is limited to pre-prepared, verified non-viable, cell-free samples and food-grade oil. No microbial culture, sample generation or unknown-isolate handling is included.

Purpose and scope

This assay estimates the 24-hour persistence of an emulsion formed by a pre-prepared, verified non-viable, cell-free sample and a selected food-grade oil. It is not a microbial cultivation or sample-generation procedure.

Principle

The emulsification index at 24 hours (E24) is the height of the visually defined emulsion layer divided by the total liquid-mixture height, multiplied by 100. E24 describes emulsion stability under the stated conditions; it is not a direct measurement of surface tension or a definitive identification of a biosurfactant.

Materials

- Pre-prepared, cell-free, institutionally verified non-viable test material from an approved source.
- One selected food-grade oil, such as olive or sunflower oil, with type and batch recorded.
- Matching uninoculated medium or matrix processed as the negative control.
- A pre-validated reference emulsifier preparation as positive assay-performance control.
- Clean transparent straight-sided capped tubes, calibrated pipettes, timer, consistent mixing equipment and a ruler or image-based measurement setup.

Controls

Control	Role
Negative matrix control	Checks whether the sample matrix alone forms an emulsion with the selected oil.
Positive reference control	Confirms that the assay system can show reproducible emulsion formation.
Matrix blank	Documents the background appearance of the aqueous matrix.

Procedure

1. Assign sample and replicate identifiers. Record source code, matrix, oil identity and batch, date and operator.

2. Bring the test material, controls and oil to a consistent room temperature. Homogenize the aqueous samples gently without introducing excessive bubbles.

3. For each replicate, combine equal volumes of the pre-prepared test material and selected food-grade oil in a clean, transparent, capped tube. Keep the ratio identical across samples and controls.

4. Prepare negative matrix, positive reference and matrix blank tubes using the same vessel format and observation plan.

5. Apply the same defined mixing setting and duration to each tube. Record the equipment and settings used.

6. Place tubes upright and undisturbed for 24 hours under the pre-defined observation conditions.

7. At 24 hours, record total liquid-mixture height and visually distinct emulsion-layer height using the same measurement convention. Photograph tubes against a consistent background where possible.

8. Use independent replicates and preserve raw measurements and images. Replicate number and biological replication must be specified in the study design.

Calculation

E24 (%) = (height of emulsion layer / total height of liquid mixture) x 100

Reporting

- Report oil identity and batch, sample matrix, mixing settings, observation conditions and exact definition of the emulsion layer.
- Present individual replicate values and an appropriate summary (for example, mean and standard deviation).
- Distinguish an emulsion layer from cream, foam or an oil layer and use the same convention for all samples.
- Interpret E24 as a screening measure of emulsification; it does not establish molecular identity, critical micelle concentration or reduction in surface tension.

References and further reading

1. Cooper DG, Goldenberg BG. Surface-active agents from two Bacillus species. Applied and Environmental Microbiology. 1987;53(2):224-229. doi:10.1128/aem.53.2.224-229.1987

2. Screening Concepts for the Isolation of Biosurfactant Producing Microorganisms. Methods in Molecular Biology / NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK6189/

3. Bio-protocol. Determination of the Emulsification Index (EI24). https://bio-protocol.org/exchange/minidetail?id=5117306&type;=30

Document status: Version 1.0. This reference should be checked against current literature, institutional requirements and the approved local method before use.