Easy Plate LS Instruction Manual code: 61800

CAUTION!
1. Do not attempt to eat this product, touch the growth area with bare hands or allow the medium to get into eyes.
2. Please make sure to read the precautions and instructions in this Instruction Manual before attempting to use the kit and exercise extreme caution when using it.

Product Description and Intended Use

Easy Plate LS is a prepared microbiological culture device made up of a waterproof sheet, a dry medium on the sheet and a transparent cover over the medium. The Easy Plate LS method is intended to enumerate or detect *Listeria* species in environment and food samples. It is compact and easy to use and reduces the total amount of waste produced during testing. Easy Plate LS is manufactured at an ISO 9001-certified site.

Contents of Product

One box of this product contains 100 sheets.
- 25 sheets per bag
- 4 bags in a box

Materials Required but Not Provided

- Pipette or Pipettor and pipette tips: Calibrated to accurately deliver 1.0 mL.
- Sterile diluent or buffer: Buffered peptone water (BPW), Butterfield's phosphate-buffered diluent (BPBD) or other diluents according to ISO 6887 or equivalent guidelines.
- Tube: With a capacity of more than 10 mL.
- Incubator

Additional materials for environmental samples
- Sterile stick swab (if required): Tipped with cotton or synthetic material (such as polyester, rayon, or polyurethane), free from inhibitory substances, with 9–10 mL swabbing broth* in a tube.
- Sterile sponge (if required): Free from inhibitory substances, with 90–100 mL swabbing broth* in a bag.

*Choose broth such as BPW or peptone water according to ISO 18593 or equivalent guidelines. If required, use a neutralizing broth such as Letheen broth (LB) or Dey-Engley broth (D/E broth).

Additional materials for food and beverage samples
- Paddle blender: e.g., Seward or equivalent.
- Sampling bag with filter

For enumeration method

No special requirements for enumeration.

For detection method
- Sterile inoculating loop
- Half-Fraser broth (HFB): Used as an enrichment broth. Other enrichment broths (e.g., buffered Listeria enrichment broth (BLEB)) may be used according to ISO 11290 or equivalent guidelines.

Instructions for Use – Enumeration Method

1. Sample preparation

Choose an appropriate sample preparation method. Refer to ISO 6887 or equivalent guidelines. The following method is an example.

1) Using a swab pre-moistened in 9-10 mL swabbing broth or sponge pre-moistened in 90-100 mL swabbing broth, sample each test area by stroking back and forth 10 times each in a vertical and horizontal direction while applying firm pressure. Return the swab to its sample tube and the sponge to its sample bag. Vortex the swab tubes and hand massage or pummel the sponge bags. This serves as the initial dilution*. For food samples, add 9 times volume of diluent to the sample and blend for up to 2 minutes.

   *Some neutralizing broths, such as D/E broth, require at least a 1:10 dilution.
2) If required, prepare decimal dilutions by transferring 1 mL of the previous dilution into 9 mL of diluent and shake 25 times.

2. Inoculation

1) Allow the bag of Easy Plate LS to reach room temperature (15-25℃). Then, remove the required number of sheets from the bag under aseptic conditions.
2) Place the sheet on a flat surface.
3) Lift the cover and place 1 mL sample suspension onto the center of the plate.
4) Lower the cover onto the sheet and allow the sample to spread evenly*.

   *If the cover sheet on the plate becomes bent, discard and replace the entire plate. A bent cover sheet will prevent uniform spreading of the sample.
5) Allow the plate to settle for 3 minutes or more on a horizontal surface. Do not tilt the sheet until solidification of the suspension is complete. Once solidification is complete, the plate can be handled normally.
6) Hold both ends of the sheet and place it into an incubator. Up to 25 plates can be stacked for space saving and convenience.

3. Incubation

Incubate the plates at 35 ± 1℃ or 37 ± 1℃ for 24 ± 1 hours.

4. Interpretation

Count all blue colonies regardless of size or intensity. The suitable range for colony counting is under 250 colonies.

5. Confirmation

If there are colonies of presumptive *Listeria* species, perform confirmation test according to ISO 11290-2 or equivalent guidelines if required. A single colony can be picked from the gel.

Troubleshooting for interpretation

a) When the entire growth area becomes blue, record the count as too numerous to count (TNTC).

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b) When the number of colonies per plate exceeds 250, for all dilutions, record the count as too numerous to count (TNTC). If an estimated count is required, count colonies within 1-3 squares (1 cm x 1 cm) printed on the cover and calculate an average. Multiplying the average number by 20 provides the estimated count since the circular growth area is approximately 20 cm^2*.

*Retesting with a higher dilution is recommended for improved accuracy.

c) When a bubble disrupts a colony so that the colony outlines the bubble, count it as one colony.

d) When a colony is spreading, count it as one colony.

e) When two or more spreading colonies appear to originate from separate sources, count each source as one colony.

f) When the sample is not clear (i.e. cloudy or dark), prepare a higher dilution.

g) If bacterial growth is insufficient, incubation may be extended up to 28 hours.

Instructions for Use – Detection Method

1. Sample preparation and enrichment

Users should choose an appropriate method for the sample preparation. Refer to ISO 6887 or equivalent guidelines. The following method is an example.

1) Using a swab pre-moistened in 9-10 mL swabbing broth or sponge pre-moistened in 90-100 mL swabbing broth, sample each test area by stroking back and forth 10 times each in a vertical and horizontal direction while applying firm pressure. Return the swab to its sample tube and the sponge to its sample bag. Vortex the swab tubes and hand massage or pummel the sponge bags. For food samples, skip this step.
2) Add 9 times volume of HFB and homogenize.
3) Incubate at 30 ± 1℃ for 25 ± 1 hours for enrichment.

2. Inoculation

1) Allow the bag of Easy Plate LS to reach room temperature (15-25℃). Then, remove the required number of sheets from the bag under aseptic conditions.
2) Place the sheet on a flat surface.
3) Lift the cover and streak the enrichment onto the plate using a loop.
4) Add 1 mL diluent onto the center of the plate.
5) Lower the cover onto the sheet and allow the diluent to spread evenly*.

*If the cover sheet on the plate becomes bent, discard and replace the entire plate. A bent cover sheet will prevent uniform spreading of the diluent.

6) Allow the plate to settle for 3 minutes or more on a horizontal surface. Do not tilt the sheet until solidification of the suspension is complete. Once solidification is complete, the plate can be handled normally.
7) Hold both ends of the sheet and place it into an incubator. Up to 25 plates can be stacked for space saving and convenience.

3. Incubation

Incubate the plates at 35 ± 1℃ or 37 ± 1℃ for 24 ± 1 hours.

4. Interpretation

If blue colonies are observed, consider them presumptive-positive. If there are numerous colonies, the entire medium is colored blue.

5. Confirmation

If *Listeria* species are presumptive-positive, perform confirmation test according to ISO 11290-1 or equivalent guidelines if required.

Precautions

1) This product is not to be used for clinical testing.
2) This product has not been tested with all possible food products, food processes, test protocols or strains.
3) The Easy Plate LS sheets are decontaminated in the manufacturing process though not sterilized.
4) Do not open the cover until just before inoculation.
5) Do not use the product after its expiry date.
6) Do not use any plates that show damage or are deformed, discolored, or show presence of foreign materials.
7) Do not expose the product to direct sunlight.
8) Use caution when lowering the cover onto the media and spreading the sample suspension. Avoid direct pressure on the plate cover and avoid causing the suspension to spill out from the growth area. If the sample suspension does spill out from the growth area, discard the plate and repeat the inoculation with a new plate.
9) Always wear safety eyewear when performing inoculations and analysis. If medium or reagents get into the eyes or mouth, flush thoroughly with water and seek medical attention.
10) Analysis needs to be performed under the control of a skilled microbiologist. Refer to ISO 7218 or equivalent guidelines.
11) Occasionally, there may be a stretched spot in the ring outside the medium area, coloration along the ring or brown-to-black particles and hollow spots may be present in the medium area, but they do not affect bacterial growth.
12) Bubble may form in the growth area after sample application, but they do not affect bacterial growth.
13) After opening the package, use the plates immediately or return unused sheets to the package, seal it, and store them according to “Storage after Opening the Packaging.”

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Otherwise, the plates may dry and curl, although this does not affect bacterial growth.

Storage and Shelf Life before Opening the Package

1) Store in the refrigerator (2-8°C). Expiry date when stored properly in unopened packaging is specified on the side of the box and the back side of the bag after the word “EXP”. The shelf life of the product under refrigerated condition (2-8°C) is 18 months after manufacturing.
2) The product can be stored in packaging 25°C for up to 14 days or 30°C for up to 5 days before opening the packaging.

Storage after Opening the Packaging

Put any unused sheets back into the packaging, fold the end of the packaging over twice, and seal with tape. The shelf life under refrigerated conditions is 3 months after opening.

Disposal

Any and all media, supplements, and reagents must be sterilized by autoclaving after use, and then disposed as industrial waste according to local laws and regulations.

Warranty

Kikkoman Biochemifa Company warrants the products to have a certain level of quality. This warranty guarantees that Kikkoman Biochemifa Company shall replace defective products should any be found. This warranty does not provide any other guarantees. Kikkoman Biochemifa Company shall not be liable for any damages, including special or consequential damages, or expenses arising directly or indirectly from the use of this product.

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