

**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 device and exercise extreme caution when using it.

**Product Description and Intended Use**

Easy Plate EC 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 EC method is intended to indicate the level of *Escherichia coli* and coliform bacteria in selected food and beverage products. It is compact, easy to use, and reduces the total amount of waste produced during testing. Easy Plate EC is manufactured at an ISO (International Organization for Standardization) 9001 certified site.

**Information of the Validation****AOAC Performance Tested Methods Certificate #031601**

In AOAC *Performance Tested Methods*<sup>SM</sup> (PTM) study, the Easy Plate EC method produced statistically similar results when compared to AOAC Official Method 966.24.

**Matrixes of the validation:**

Raw beef, raw pork, raw frozen pork, raw lamb, raw salmon, Frankfurter sausage and cooked ham.

**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\***

- Incubator (35 ± 1°C)
- Stomacher or Blender
- Sampling bag
- Pipette or Pipettor and pipette tips
- Butterfield's phosphate-buffered diluent or appropriate diluents according ISO6887

\* See section “**Specific Instructions for Validated Methods**” for specific requirements.

**Instructions for Use****1. Sample preparation**

User should choose an appropriate method for the sample preparation. Refer to ISO 6887, FDA Bacteriological Analytical Manual (BAM) or other regulatory guidelines. See section “**Specific Instructions for Validated Methods**” for specific requirements.

The following method is an example.

- 1) Weigh 50 g test portions into a blender jar.
- 2) Add 450 mL Butterfield's phosphate-buffered diluent (BPD) and blend for up to 2 minutes.
- 3) Prepare all decimal dilutions with 90 mL BPD plus 10 mL previous dilution.

- 4) Shake 25 times.

**2. Inoculation**

- 1) Allow the bag of Easy Plate EC to reach room temperature (15-25°C). Then, remove required number of the 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.

**3. Incubation**

Incubate the plate(s) at 35 ± 1°C for 24 ± 1 hours. Up to 25 plates can be stacked for space saving and convenience. See section “**Specific Instructions for Validated Methods**” for specific requirements.

**4. Interpretation**

Medium contains two kinds of colorimetric enzymes substrate, Magenta-Gal and X-Gluc. Count pink/red purple colonies for non-*E. coli* coliforms and navy blue/blue purple colonies for *E. coli*. The range of enumeration is 1-250. See section “**Specific Instructions for Validated Methods**” for specific requirements.

**Troubleshooting for counting colonies:**

- a) Not every *Escherichia coli* forms navy blue/blue purple colony.
- b) *Escherichia coli* O157 is detected as non-*E. coli* coliform and forms red purple colonies because it does not produce β-glucuronidase.
- c) *Aeromonas hydrophila* and some of *Serratia* species are detected as non-*E. coli* coliform and form red purple colonies because they have ability to produce β-galactosidase.
- d) When the entire growth area becomes colored, record the count as too numerous to count (TNTC).
- e) When a bubble disrupts a colony so that the colony outlines the bubble, count it as one colony.
- f) When a colony spreads, count it as one colony.
- g) When two or more spreading colonies appear to originate from separate sources, count each source as one colony.
- h) When the sample is not clear (i.e. cloudy or dark), prepare a higher dilution.

- i) When the entire growth area becomes colored due to food components involving the chromogenic reaction, prepare a higher dilution.

## 5. Colony isolation

Lift the cover and pick a single colony from the gel.

## Specific Instruction for Validated Methods

### AOAC-PTM

#### 1. Sample preparation

Use BPD for diluents.

#### 2. Incubation

Incubate the plate(s) at  $35 \pm 1^\circ\text{C}$  for  $24 \pm 1$  hours.

#### 3. Interpretation

The range of enumeration is 1-250. 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<sup>2</sup>.

## 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 EC 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 Good Laboratory Practices\* or ISO 7218.

\*Ex) U.S. Food and Drug Administration. Code of Federal Regulations, Title 21, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.

## Storage

Store in the refrigerator (2-8°C).

The product can be stored in the product packaging up to 25°C for up to 14 days or up to 30°C for up to 5 days before opening the packaging. Storage at 25°C and 30°C have not been validated under AOAC Research Institute PTM 031601.

## 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 condition is 3 months after opening. This period has not been validated under AOAC Research Institute PTM 031601.

## Shelf Life

Expiry date 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 is 18 months after manufacturing if it is stored properly in unopened packaging.

## 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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