

Enumeration of Total *E. coli* and *E. coli* O157:H7

AOAC Method
#997.11



Intended Use

Using SD-39 Agar, the ISO-GRID Membrane Filter System is used to provide a method for enumeration of *E. coli* and *E. coli* O157:H7.

The Test

The ISO-GRID system is based on the principle of hydrophobic grid membrane filtration. A sample's total *E. coli* and *E. coli* O157:H7 is enumerated through the use of a unique membrane filter containing a grid of 1,600 squares. First, a diluted sample is filtered through a 5 µm stainless steel prefilter to eliminate any food particles present. The sample is then filtered through a hydrophobic membrane, and the membrane is placed on SD-39 Agar. SD-39 Agar is specifically formulated to enumerate a sample's total *E. coli* and *E. coli* O157:H7.

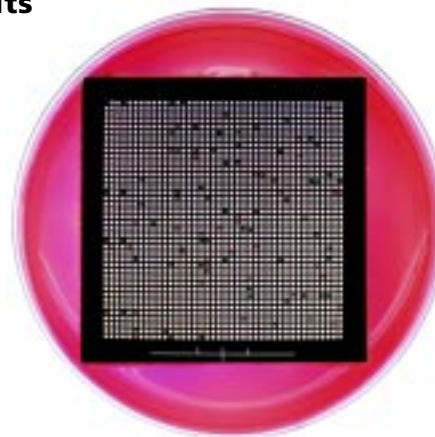
After incubation the membrane filter is examined, and all squares containing one or more pink and/or green colonies are counted. The total number of positive squares is converted to the corresponding most probable number (MPN) using one of the methods described in the ISO-GRID Methods Manual, and the total *E. coli* MPN per gram is calculated. A presumptive *E. coli* O157:H7 MPN per gram is calculated by counting all the squares containing one or more pink colonies and following the same procedure. The presence of *E. coli* O157:H7 can be confirmed using the confirmation procedures described in the ISO-GRID Methods Manual.

Test Procedure

1. Prepare a sample homogenate in a specified diluent.
2. Filter 1 mL of the homogenate through the prefilter and ISO-GRID membrane filter.
3. Place the membrane filter on the surface of SD-39 Agar.
4. Incubate inverted plate for 24 hours at 44.5°C.

Refer to ISO-GRID Methods Manual for complete instructions, and other available test methods.

Results



SD-39 Agar

Proteose Peptone SM47	5 g/L
Yeast Extract	3 g/L
Sodium Chloride	5 g/L
L-Lysine Monohydrochloride	10 g/L
Dextrose	2.5 g/L
Sorbitol	20 g/L
Magnesium Sulfate 7-Hydrate	1.5 g/L
Monensin	0.038 g/L
Sodium Glucuronate	0.5 g/L
Novobiocin	0.0075 g/L
Phenol Red	0.12 g/L
X-glucose	0.05 g/L
Agar	15 g/L
Final pH: 7.2 ± 0.2	

See reverse side for a complete list of ISO-GRID advantages and products.

ISO-GRID Total *E. coli* and *E. coli* O157:H7 Testing Products Available From Neogen

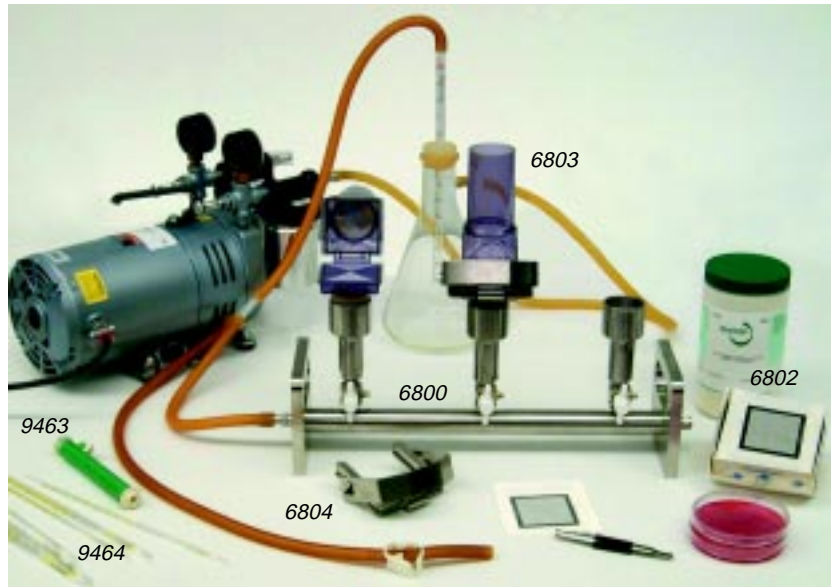
Prod#.	Description
6810	1-Place Filtration Manifold
6800	3-Place Filtration Manifold
6801	6-Place Filtration Manifold
6802	Membrane Filters (100)
6803	Filtration Unit
6804	Filtration Unit Clamp
6805	Methods Manual
6907E, F	SD-39 Agar

ISO-GRID™ Advantages

- **APPROVALS.** ISO-GRID methods for *E. coli* O157:H7, *Salmonella*, yeast and mold, coliform / *E. coli*, and total aerobic plate count are AOAC Official Methods, and recognized by the FDA. Other ISO-GRID methods hold numerous approvals.
- **SPEED.** ISO-GRID methods are fast. ISO-GRID yeast and mold detection takes just 2 days, start to finish. The combined coliform / *E. coli* test is complete in just 24 hours. A *Salmonella* negative screen is complete in as little as 24 hours.
- **VERSATILITY.** All ISO-GRID analyses use the same basic procedure, simplifying work flow and training.
- **COUNTING RANGE.** A single ISO-GRID membrane filter has a counting range spanning greater than three 10-fold dilutions. Just **one filter** eliminates the need for both multiple sample dilutions and duplicate plating.
- **REPRODUCIBILITY.** AOAC-sponsored studies have repeatedly demonstrated that ISO-GRID methods produce consistent and highly reproducible results. Published references are available.

Equipment

- 6810 1-Place Filtration Manifold
- 6800 3-Place Filtration Manifold
- 6801 6-Place Filtration Manifold
- 6802 Membrane Filters (100)
- 6803 Filtration Unit
- 6804 Filtration Unit Clamp
- 6805 Methods Manual
- 6806 Filtration Unit Base
- 6807 Filtration Unit Funnel
- 6808 ISO-GRID Starter Kit - 200 membrane filters, 4 filter units, 2 filter unit clamps, Methods Manual
- 6809 Paddle Bags (500)
- 9463 Pipettor (100 µL-1 mL)
- 9464 Pipette Tips
- 9735 Incubator w/thermometer



Enzyme Products

- 6850E Papain
- 6851E APUG (alkaline protease)
- 6852E AMG (amyloglucosidase from rhizopus mold)
- 6853E Cellulase
- 6854E Hemicellulase

Culture Media

- 6900E Bacteriological Agar
- 6901E EF-18 Agar - for *Salmonella* detection
- 6902E LMG Agar - for *E. coli* / coliform enumeration
- 6903E BMA Agar - for confirmation of *E. coli*
- 6904E YM-11 Agar - for enumeration of yeasts and molds
- 6905E TSAF Agar - for total bacterial count
- 6906E LM-137 Agar (125 g + supplement pool) - for *Listeria* detection
- 6907E SD-39 Agar - for enumeration of *E. coli* / *E. coli* O157:H7
- 6909E Tetrathionate Broth Base - *Salmonella* Enrichment Broth
- 6910E SCCRAM Broth - *Salmonella* Enrichment Broth
- 6912E Chlortetracycline-HCL - antibiotic supplement for YM-11 Agar



6801 6-Place Filtration Manifold; 6810 1-Place Filtration Manifold

Equipment and accessories required but not available through Neogen

- | | | |
|---|-----------------|----------------|
| • Side-arm 1000 mL flask | • Autoclave | • Stomacher |
| • Purified water | • Vacuum pump | • Water bath |
| • Forceps (sterile) | • Vacuum tubing | • Petri dishes |
| • Sterile diluent (Butterfield's phosphate buffer, peptone / Tween 80, peptone water) | | |



9735 Incubator w/thermometer



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