

Instructions for Use

COMPACT DRY™ ETB

Cat. no. ETB100	Compact Dry™ ETB, 60x75mm Tray with 10x60mm Well	100 trays/box
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INTENDED USE

Hardy Diagnostics Compact Dry™ ETB is a ready-to-use test method recommended for the isolation and enumeration of organisms belonging to the family *Enterobacteriaceae* in raw materials, finished products, or on environmental surfaces pertaining to food and related industries.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

The family *Enterobacteriaceae* is a large, diverse family of gram-negative bacteria that includes a wide variety of harmless symbionts and recognized pathogens. Of these, *Salmonella*, *Escherichia coli*, and *Shigella* are well-documented food-borne pathogens, and *Salmonella* alone was responsible for 35% of all food-borne illness-related hospitalizations and 28% of food-borne illness-related deaths in the United States in 2011.^(1, 4) Thus, the detection of these potentially pathogenic species is essential in ensuring food safety for consumers.

Compact Dry™ ETB is a ready-to-use chromogenic medium for detecting and enumerating *Enterobacteriaceae* that contains dehydrated culture media and a cold water-soluble gelling agent in a non-woven cloth matrix. The medium is instantly hydrated when inoculated with a sample, and capillary action diffuses the sample evenly over the matrix to form a gel within seconds. *Enterobacteriaceae* colonies grown on Compact Dry™ ETB turn red or purple due to a chromogenic indicator included in the media.

Compact Dry™ ETB is comparable to other dry film test methods for the detection and enumeration of *Enterobacteriaceae*.⁽⁶⁾ Compact Dry™ ETB is AOAC validated (AOAC no. 012001), NordVal validated (NordVal no. 034), and MicroVal validated (no. MV0806-002LR). The ready-to-use trays save space and greatly reduce the time needed to perform microbiological testing. Compared to other commonly used culture systems, Compact Dry™ has a longer shelf life, can be stored at room temperature, does not require manual sample spreading, is rigid, stackable and easy to label, and allows for direct colony picking for further subculture.

FORMULA

Compact Dry™ ETB contains dehydrated culture media with glucose, a gelling agent, and a chromogenic indicator to facilitate the identification and differentiation of *Enterobacteriaceae* from other types of organisms.

Final pH 7.0 +/- 0.2 at 25°C.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 1-30°C. away from direct light. Media should not be used if there are any signs of deterioration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect

from light, excessive heat, moisture, and freezing. If foil pouch is opened and not all plates are used, return remaining plates to pouch and reseal until next use. Opened packages should be used as soon as possible.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

Prior to Use: Refer to listed references for appropriate methods of collection, preparation, and dilution of samples under investigation. ⁽¹⁻⁵⁾

For environmental samples or to test uneven surfaces of equipment, swab surface of test area with [EnviroTrans™](#) (e.g. Cat. no. SRK05 or SRK35).

For raw material and food testing, prepare and dilute samples using an appropriate diluent such as [Dilu-Lok™ II](#) (e.g. Cat. no. D590 or D599).

General Dilution Guidelines:

For Making 1:10 Serial Dilutions

1. Using a sterile pipet or scoop, aliquot 10ml or 10gm of test sample to a 90ml pre-filled Dilu-Lok II™ dilution vial to yield a 1:10 dilution. Mix thoroughly.
2. From the 1:10 dilution vial in step 1, use a fresh sterile pipet and aliquot 10ml from this dilution vial into a second 90ml pre-filled Dilu-Lok II™ vial to yield a 1:100 dilution. Mix thoroughly.
3. Continue aliquoting 10ml dilutions into 90ml pre-filled Dilu-Lok II™ vials until the desired concentration of test sample is achieved. Each subsequent dilution increases by a factor of 10. A separate sterile pipet should be used with each dilution. Each subsequent dilution increases by a factor of 10. A separate sterile pipet should be used with each dilution.

For Making 1:100 Serial Dilutions

1. Using a sterile pipet or scoop, aliquot 1ml or 1gm of test sample to a 99ml pre-filled Dilu-Lok II™ dilution vial to yield a 1:100 dilution. Mix thoroughly.

2. From the 1:100 dilution vial in step 1, use a fresh sterile pipet and aliquot 1ml from this dilution vial into a second 99ml pre-filled Dilu-Lok II™ vial to yield a 1:10,000 dilution. Mix thoroughly.
3. Continue aliquoting 1ml dilutions into 99ml pre-filled Dilu-Lok II™ vials until the desired concentration is achieved. Each subsequent dilution increases by a factor of 100. A separate sterile pipet should be used with each dilution.

Method of Use Direct Inoculation:

1. Remove the set of four trays from the foil pouch and separate each individual tray by gently bending along the connecting edge until each tray snaps free. Alternatively, if setting up a dilution series of the same sample, trays can be left connected to facilitate reading similar samples. Trays that are not used immediately should be resealed in the foil pouch. Refer to the "Storage and Shelf Life" section for proper storage of unused trays.
2. Remove the lid of the tray using two fingers to hold down one end of the lid and the thumb to lift the opposite end. Lids are easier to remove using a "peel back" method as opposed to a "pull off" method.
3. Inoculate by pipetting 1ml of sample directly to the center of a dry tray well, being careful not to touch the surface of the matrix with the pipet tip. Once dispensed, the sample will automatically diffuse across the surface by capillary action to form a gel; manual spreading of the inoculum is discouraged. Remember to account for the sample inoculum when calculating the dilution series.
4. Replace the lid and label the tray with appropriate information, including the sample dilution factor.
5. Invert the tray and incubate, upside down with the medium on top, at 35-37° C for 24 hours. NOTE: Use the appropriate temperature/time designation according to the legal specification of the prescribed food analysis regulation.
6. Count colonies using the Hardy Diagnostics Wizard™ Compact Dry™ plate reader (Cat. no. CDR1) designed exclusively for use with Compact Dry™. See the Wizard™ Compact Dry™ instruction manual. Alternatively, colonies can be counted when illuminated from the backside of the tray to calculate CFU/ml using the Scan® 100 colony counter (Cat. no. 435000) or comparable back lighting. If the colony count is high, use the 1cm x 1cm molded grid on the back of the tray to assist in colony counting. Use a sheet of white paper with gridded lines to diffuse the light if the molded grids in the tray are difficult to visualize with a light box.

Method of Use Membrane Filtration:

1. Remove the lid on a Compact Dry™ plate and pipette 1.0ml of sterile purified water into the middle of the plate to activate the matrix just before use.
2. Using a membrane filtration set-up, filter 100-250ml of a water sample under reduced pressure through a sterile 0.45µm membrane filter.
3. Keep the set-up running and rinse the inside of the filter funnel using 20-30ml of sterile purified water. Repeat this step two to three times to ensure all of the sample has been filtered through the membrane.
4. Remove the membrane from the funnel using sterile forceps (e.g. Cat. no. 800000) and apply it filter side up (trap side away from the matrix) to the surface of the pre-moistened Compact Dry™ plate. Gently press the membrane onto the surface, making sure to remove bubbles so the membrane is completely flush and centered on the matrix.
5. Replace the lid and incubate the tray right-side-up using the information outlined above.
6. Count colonies and record results as outlined above.

INTERPRETATION OF RESULTS

After incubation, read trays using the Wizard™ Compact Dry™ plate reader (Cat. no. CDR1) or read colonies against a white or illuminated background such as with the Scan® 100 colony counter (Cat. no. 435000) or comparable back lighting.

Enterobacteriaceae colonies will be red or purple due to utilization of the chromogenic indicator in the medium. Most colonies grown will be red or purple. Un-pigmented or otherwise colored colonies are indicative of other gram-negative bacteria. Count all red or purple colonies to obtain the total *Enterobacteriaceae* count. Count all colonies, regardless of pigmentation, to obtain the total gram-negative count. The growth area is 20cm². If the colony count is high, the total count can be obtained by multiplying the average number of colonies observed in one 1cm x 1cm square grid by 20.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

During inoculation, do not touch the surface of medium and be careful to avoid any contamination by airborne microorganisms.

During incubation, keep cap tight on plates to avoid any possible dehydration.

A dilution may be needed when the sample has a dark color.

When the sample is viscous (thick), pipetting the sample on several points on a plate or an additional dilution may be needed for an even suspension.

When the sample contains an enzyme, it may react with the enzyme substrate in the dry sheet and affect the color.

If the nature of sample does affect the reaction of the medium, inoculate only after the factor is eliminated by means of dilution and other techniques (e.g. samples with high viscosity, colored, reactive with chromogenic substrate, and with a high or low pH).

It is recommended to use a stomacher and filter homogenized sample afterwards to eliminate carry over of tiny particles of foodstuff onto the surface of the medium.

Some colonies may not be clearly red or purple colored.

Counting colonies may be difficult against a dark background. For best results, count colonies using the Hardy Diagnostics Wizard™ Compact Dry™ plate reader (Cat. no. CDR1) or with the tray held against a white or illuminated background such as with the Scan® 100 colony counter (Cat. no. 435000).

If using a light box, molded grid lines in the tray, or colonies, may be difficult to view due to excessive brightness. Diffuse the light using a sheet of white, gridded (1cm x 1cm) paper underneath the tray to facilitate colony counting.

Colonies are not distinguishable on trays if concentrations are above 100 CFU/ml, as high colony counts will result in the whole surface becoming colored. The sample should be diluted to a concentration of less than 100 CFU/ml for best use.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs such as EnviroTrans™, applicator sticks, scoops, dilution buffers such as Dilu-Lok™ II, other culture media, Wizard™ Compact Dry™ plate reader (Cat. no. CDR1), Scan® 100 colony counter (Cat. no. 435000), incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

End users can anticipate the following typical performance characteristics when testing with CompactDry™.

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Escherichia coli</i> ATCC® 25922	J	24±2hr	35-37°C	Aerobic	Growth; red colonies
<i>Salmonella enterica</i> var. Typhimurium ATCC® 14028	J	24±2hr	35-37°C	Aerobic	Growth; red colonies
<i>Klebsiella pneumoniae</i> ATCC® 13883	J	24±2hr	35-37°C	Aerobic	Growth; red colonies
<i>Enterococcus faecalis</i> ATCC® 29212	J	24±2hr	35-37°C	Aerobic	No growth

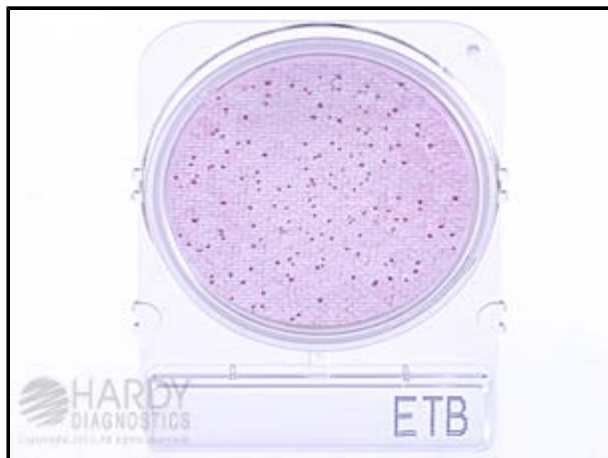
* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

USER QUALITY CONTROL

Check for signs of contamination and deterioration. Users of commercially prepared culture media may be required to perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction (where applicable). See the following reference for more specific information. ⁽¹⁻⁵⁾

PHYSICAL APPEARANCE

Compact Dry™ ETB should appear dry, free of particles, and light yellow to light pink in color.



Escherichia coli (ATCC® 25922) colonies growing on Compact Dry ETB (Cat. no. ETB100). Incubated aerobically for 24 hours at 35°C.

REFERENCES

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<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm>
5. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*. APHA, Washington, D.C.
6. Teramura, Hajime, Shingo Mizuochi, and Hidemasa Kodaka. "Evaluation of the dry sheet medium (Compact Dry ETB (R)) for enumerating Enterobacteriaceae in meat samples." *Biocontrol science* 17.3 (2011): 149-154.
7. Association of Official Analytical Communities. *Official Methods of Analysis*. AOAC, Washington, D.C.

ATCC is a registered trademark of the American Type Culture Collection.
Compact Dry™ is a trademark of Shimadzu Diagnostics Corporation. Ltd.
Scan is a registered trademark of Interscience for Microbiology.

AOAC approval no. 012001

MicroVal approval no. MV0806-002LR per ISO 16140:2003

NordVal approval no. 034

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