

# Instructions for Use

## COMPACT DRY™ TCR

<a href="#">Cat. no. 54088</a>	Compact Dry™ TCR, 60x75mm Tray with 10x60mm Well	240 trays/box
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### INTENDED USE

Hardy Diagnostics Compact Dry™ TCR (Total Count Rapid) is a ready-to-use test method recommended for the enumeration of mesophilic total aerobic bacterial counts in food commodities, including raw meat and fish, bagged pre-washed lettuce and vegetables, and pasteurized whole milk, heavy cream, and nonfat dry milk, using traditional or rapid enumeration methods.

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

### SUMMARY

Microbiological testing is a common practice in food and related industries to ensure product quality and a high level of environmental control. Foods, beverages, and similar finished products are usually not sterile. However, in order to ensure they are safe for human consumption, maximum cell load numbers for the finished product, as well as for raw materials used to produce them, have been established. Procedures for determining aerobic plate count (APC) of foods were developed by the Association of Official Analytical Communities (AOAC) and the American Public Health Association (APHA).<sup>(2-4, 5)</sup> Conventional plate count methods for examining frozen, chilled, precooked, or prepared foods and the automated spiral plate count method for the examination of foods and cosmetics are outlined in FDA *Bacterial Analytical Manual*.<sup>(1)</sup>

Compact Dry™ TCR is a ready-to-use chromogenic medium for performing total viable aerobic bacterial counts by the traditional or rapid method. The medium is specifically formatted to allow most microorganisms to grow more quickly than the standard Compact Dry TC. Compact Dry™ TCR 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. Colonies grown on Compact Dry™ TCR turn red/pink due to the redox indicator, triphenyltetrazolium chloride (TTC).

Compact Dry™ TCR is comparable to other dry film test methods and to the spiral plate method.<sup>(6)</sup> Compact Dry™ TCR is AOAC validated (AOAC no. 082201) and 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™ TCR contains dehydrated culture media, a gelling agent, and the redox dye, 2,3,5-triphenyl tetrazolium chloride (TTC), to facilitate differentiation of colony growth.

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. <sup>(1-5)</sup>

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 Viable Food Counts:**

##### Viable Count in Solid Foodstuff (except dairy):

1. Weigh 50gm of sample and add to 450ml of Butterfield's Phosphate Buffer ([Cat. no. U150](#)). Homogenize by blender for 2 minutes.
2. After sample preparation, follow the steps under Method of Direct Inoculation for further testing.

##### Viable Count in Dairy Products (pasteurized whole milk & heavy cream, as well as nonfat dry milk):

1. Weigh 11gm or 11mL of sample and add to 99ml of Butterfield's Phosphate Buffer. Homogenize by blender for 2 minutes.
2. After sample preparation, follow the steps under Method of Direct Inoculation for further testing.

#### **Method of Use Swab Samples:**

1. Using an area sampling template ([Cat. no. T2905C](#)) or similar test method for enumeration, obtain a sample using a sterile swab pre-moistened with an appropriate diluent (e.g. saline) or neutralizing buffer. Perform sample serial dilution in a buffer to obtain a concentration less than 100cfu/plate.

#### **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 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  $35 \pm 1$  °C for foodstuff except dairy, or  $35 \pm 1$  °C or  $32 \pm 1$  °C for pasteurized whole milk, heavy cream and nonfat dry milk for  $48 \pm 3$  hours. For more rapid measurements, incubate at  $35 \pm 1$  °C for foodstuff except dairy, or  $35 \pm 1$  °C or  $32 \pm 1$  °C for pasteurized whole milk, heavy cream, and nonfat dry milk for  $24 \pm 2$  hours. NOTE: Use the appropriate temperature/time designation according to the legal specification of the prescribed food analysis regulation.

6. Count colonies from the backside of the plate using the Hardy Diagnostics Wizard™ CompactDry™ plate reader (Cat. no. CDR2) 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 backlighting. 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 whitepaper with gridded lines to diffuse the light if the molded grids in the tray are difficult to visualize with a light box.

## INTERPRETATION OF RESULTS

After incubation, read trays using the Wizard™ CompactDry™ plate reader (Cat. no. CDR2) or read colonies against a white or illuminated background such as with the Scan® 100 colony counter (Cat. no. 435000) or comparable back lighting.

Most colonies will be red due to reduction of TTC. Count all colonies, regardless of pigmentation, to obtain the total aerobic count. The growth area is 20cm<sup>2</sup>. 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

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 the 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 carryover of tiny particles of foodstuff onto the surface of the medium.

Since some microorganisms may not reduce TTC, some colonies may not develop a red/pink color. Count all colonies present, regardless of pigmentation.

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. CDR2) 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. CDR2), Scan® 100 colony counter (Cat. no. 435000), blender or stomacher, 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>Bacillus subtilis</i> ATCC® 6633	J	48hr	35-37°C	Aerobic	Growth;red colonies
<i>Escherichia coli</i> ATCC® 8739	J	48hr	35-37°C	Aerobic	Growth; red colonies
<i>Klebsiella pneumoniae</i> ATCC® 13883	J	48hr	35-37°C	Aerobic	Growth;red colonies
<i>Pseudomonas aeruginosa</i> ATCC® 9027	J	48hr	35-37°C	Aerobic	Growth;red colonies
<i>Staphylococcus aureus</i> ATCC® 6538	J	48hr	35-37°C	Aerobic	Growth;red colonies

\* 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. <sup>(1-5)</sup>

## PHYSICAL APPEARANCE

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

## REFERENCES

1. Association of Official Analytical Communities. *Official Methods of Analysis*. AOAC, Washington, D.C.
2. American Public Health Association. *Standard Methods for the Examination of Dairy Products*. APHA, Washington, D.C.
3. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*. APHA, Washington, D.C.
4. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. Arlington, VA  
<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. Kodaka, Hidemasa, et al. 2005. "Comparison of the compact dry TC method with the standard pour plate method (AOAC Official Method 966.23) for determining aerobic colony counts in food samples: performance-tested method." *Journal of AOAC International* 88(6): 1702-1713.

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AOAC approval no. 082201

IFU-000815[A]

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