

BD Bio-Bag™ Environmental Chamber **(Type A) Multi-Plate**

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INTENDED USE

Bio-Bag™ (Type A) Multi-Plate is designed to provide an anaerobic environment suitable for the isolation of obligate or facultative anaerobic bacteria as well as to permit visualization without disrupting the gaseous atmosphere. It is sized to hold three standard 100-mm Petri dishes.

SUMMARY AND EXPLANATION

Recognition of anaerobic organisms as causative agents of disease is important to microbiologists and clinicians.^{1,2} New techniques in isolation, identification, and susceptibility testing have provided a better understanding of these fastidious organisms.^{2,3} Proper collection of specimens to prevent contamination by normal flora and to provide adequate material for testing, as well as appropriate transport of specimens to the laboratory, are critical in isolation and subsequent identification of pathogens.^{2,4}

By definition, anaerobic organisms require reduced levels or the absence of oxygen.^{2,5,6} The **Bio-Bag** (Type A) Multi-Plate system, when directions are followed, provides a rapid reduction of atmospheric oxygen. Studies have shown levels of oxygen to be less than 0.1% at 1 h through 72 h. A carbon dioxide concentration of 8.0% to 12.0% is produced within 1 h to enhance the growth of certain organisms.

Bio-Bag (Type A) Multi-Plate is a transparent, individual, disposable environmental chamber that contains a gas generator/resazurin oxygen indicator, consisting of two tablets of potassium borohydride and sodium bicarbonate and an ampule of hydrochloric acid (2.7 N), two catalyst cups containing palladium catalyst, and an indicator containing an ampule of resazurin. When the **Bio-Bag** (Type A) Multi-Plate containing all three components has been properly heat-sealed and the generator has been activated, an anaerobic environment will be created. The oxygen reduction indicator, resazurin, monitors the oxygen level.

Transparency of the **Bio-Bag** (Type A) Multi-Plate envelope allows macroscopic observation of each culture without disturbing the anaerobic atmosphere. Protection from exposure to oxygen is assured for the more fastidious organisms. As some anaerobic organisms exhibit colonies in 24 h, identification and antimicrobial susceptibility procedures can be initiated as soon as visible growth is observed.

Because of the individuality of this system, specimens can be set up as they arrive in the laboratory.

PRINCIPLES OF THE PROCEDURE

An anaerobic atmosphere is achieved in each sealed **Bio-Bag** (Type A) Multi-Plate system. A self-contained generator/resazurin indicator consists of an ampule of a weak hydrochloric acid solution and two gas-generating tablets. When the ampule is crushed, the acid reacts with the tablets, resulting in the formation of a mixture of gases including hydrogen and carbon dioxide. Hydrogen, in the presence of the palladium catalyst, quickly reacts with the atmospheric oxygen in the bag to form water. Removal of oxygen is indicated by the change in color, pink to colorless, of the resazurin indicator included with the system.

REAGENTS

Each **Bio-Bag** (Type A) Multi-Plate system consists of:

- 1 Gas-Impermeable Environmental Chamber
- 1 Gas Generator/Indicator (gas generator containing two tablets of potassium borohydride and sodium bicarbonate and a 1.0-mL ampule of 2.7 N hydrochloric acid with anaerobic indicator containing a 0.5-mL ampule of 1% resazurin)
- 2 Palladium Catalyst Containers

Warnings and Precautions

For *in vitro* Diagnostic Use.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

WARNING: HYDROGEN IS GENERATED. THIS GAS IS FLAMMABLE AND MAY BE EXPLOSIVE. AVOID EXPOSURE TO SPARKS OR FLAME.

Do not use generator if it appears damaged or previously activated.

Do not allow generator to come in contact with water prior to use. Store in tightly closed bag with desiccant to assure integrity of the generator tablet.

Do not activate generator until **Bio-Bag** (Type A) Multi-Plate has been properly sealed. Repeat procedure if indicator change is not observed within 2 h after generation process.

Do not use **Bio-Bag** (Type A) Multi-Plate anaerobic generator/indicator with any other **Bio-Bag** system.

Do not use more than three 100-mm Petri dishes in the Multi-Plate system.

Storage Instructions

Store at room temperature 15 – 30°C. Store in tightly closed bag with desiccant to assure integrity of the generator tablet.

PROCEDURE

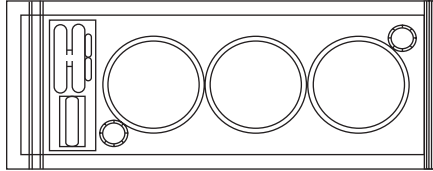
Materials Provided: Bio-Bag (Type A) Multi-Plate System

Materials Required But Not Provided: Heat sealer, Incubator, media of choice in 100-mm Petri dish

Test Procedure

1. Place one catalyst cup in bottom of bag.
2. Place up to three 100-mm plates of inoculated media in tandem in the **Bio-Bag** (Type A) Multi-Plate, right side up. (One or two API™-20A strips or one or two anaerobic MIC plates may be substituted for three Petri dishes.)
3. Place the gas generator/anaerobic indicator in the bag perpendicular to the plates and at the open end of the bag. The tablets should be closer to the sealed end of the bag.
4. Place second catalyst cup in the bag at the open end of the bag. See illustration for proper placement of components.

Recommended Positioning



5. Heat-seal the bag carefully so that it is airtight. It is recommended that two heat seals, parallel and about 2 cm apart, be made at the top of the bag.
6. Crush the indicator ampule. To do so, push with thumb against the ampule of resazurin within the **Bio-Bag**. Allow solution from crushed ampule to saturate pledget beside the indicator. A pink color indicating transient aerobic conditions will develop in approximately 1 min.
7. Crush the generator ampule. To do so, push with thumb against the ampule of hydrochloric acid within the **Bio-Bag**. The dilute hydrochloric acid will be observed flowing from the generator ampule to the two gas generator tablets; gas will be seen to evolve from the tablets. Elevate the sealed end slightly (approximately 1 in.) to allow liquid to flow into tablet chamber.
8. The bag must remain in a flat position for about 60 s until all gas is evolved.

NOTE: If several bags are being set up at the same time, separate **Bio-Bags** to prevent accumulation of heat from the catalyst.

9. Check for gradual dissipation of color in indicator's polyester pledget to confirm anaerobiosis.
10. Place sealed **Bio-Bag** (Type A) Multi-Plate in incubator at desired temperature.
11. It is recommended that the indicator be rechecked at the end of 2 h. If the indicator color has not fully dissipated in 2 h, the proper atmosphere has not been generated.

User Quality Control

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

A stock strain of an anaerobic organism should be tested in the **Bio-Bag** (Type A) Multi-Plate system periodically to assure adequate conditions for recovery and characteristic morphology.

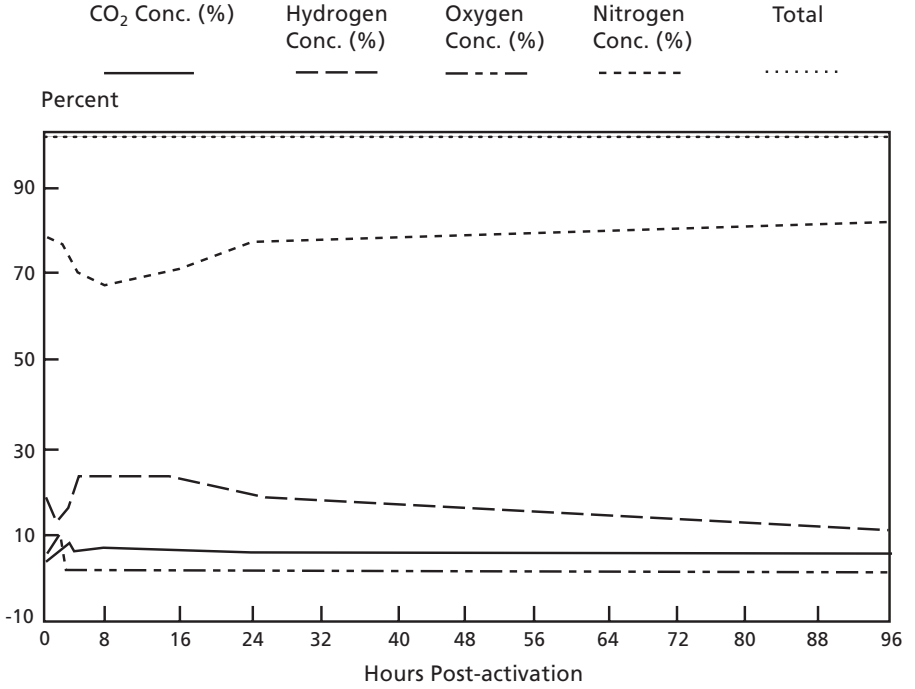
LIMITATIONS OF THE PROCEDURE

- Exposure to oxygen prior to insertion into the **Bio-Bag** (Type A) Multi-Plate may be harmful to some fastidious anaerobic pathogens.
- Tablets in the generator may deteriorate if exposed to moisture. Care must be taken to store unused generators in closed storage bag with desiccants supplied.

EXPECTED RESULTS AND PERFORMANCE CHARACTERISTICS

Studies have shown **Bio-Bag** (Type A) Multi-Plate to provide a suitable environment for the isolation of facultative and obligate anaerobic bacteria.

Three-Plate Bio-Bag System Gas Concentrations



Results:
Average percentage of 8 samples

Summary of colony count results using the Bio-Bag (Type A) Multi-Plate and an anaerobic glove box with 20 strains of various microorganisms

| Strain Number | CDC Identity of Strain | Average colony count log ₁₀ (CFU/mL) | | | | | |
|---------------|---|---|-----------|----------|-----------|---------|-----------|
| | | 24 hours | | 48 hours | | 5 days | |
| | | Bio-Bag | Glove Box | Bio-Bag | Glove Box | Bio-Bag | Glove Box |
| A6609 | <i>Bacteroides gingivalis</i> | <5.0 | <5.0 | 8.3 | 8.0 | 8.8 | 8.7 |
| 14371 | <i>Fusobacterium nucleatum</i> | 6.3 | 7.1 | 7.1 | 7.4 | 7.4 | 7.5 |
| 5164 | <i>Fusobacterium necrophorum</i> | 8.9 | 8.9 | 8.9 | 8.9 | 8.8 | 8.9 |
| 8178 | <i>Fusobacterium mortiferum</i> | 6.1 | 7.0 | 7.1 | 7.1 | 7.2 | 7.1 |
| 15689 | <i>Peptostreptococcus anaerobius</i> | 6.8 | 6.3 | 7.0 | 6.6 | 6.9 | 7.4 |
| 16208 | <i>Peptostreptococcus asaccharolyticus</i> | >7.5 | 8.3 | 8.2 | 8.6 | 8.3 | 8.5 |
| 17785 | <i>Peptostreptococcus magnus</i> | >8.5 | >8.5 | 8.0 | 8.5 | 8.2 | 8.3 |
| 14337 | <i>Clostridium sordellii</i> | 6.7 | 7.7 | 6.0 | 7.6 | 6.6 | 7.6 |
| A1494 | <i>Clostridium difficile</i> | <5.0 | 7.5 | 5.4 | 7.5 | 5.4 | 7.5 |
| 8179 | <i>Clostridium ramosum</i> | >7.5 | 7.9 | 7.8 | 7.9 | 7.7 | 7.9 |
| 14369 | <i>Propionibacterium acnes</i> | <5.0 | <5.0 | <5.0 | >7.5 | 9.4 | 9.4 |
| 14702 | <i>Bifidobacterium eriksonii</i> | 8.5 | 8.1 | 8.6 | 8.8 | 8.3 | 8.6 |
| A5723 | <i>Actinomyces israelii</i> | <5.0 | <5.0 | <5.0 | <5.0 | 9.2 | 9.3 |
| 14375 | <i>Arachnia propionica</i> | 8.1 | 8.6 | 8.2 | 8.8 | 9.0 | 9.1 |
| A6153 | <i>Actinobacillus actinomycetemcomitans</i> | 8.1 | <5.0 | 8.3 | 7.3 | 8.3 | 7.6 |
| A3563 | <i>Campylobacter sputorum</i> | >7.5 | >7.5 | 8.9 | 9.1 | 9.0 | 9.1 |
| A7115 | <i>Campylobacter concisus</i> | <5.0 | <5.0 | 7.8 | >8.5 | 8.1 | 8.5 |
| A7116 | <i>Campylobacter rectus (Wolinella recta)</i> | <5.0 | <5.0 | <5.0 | 7.8 | 6.8 | 7.8 |
| A4050 | <i>Wolinella succinogenes</i> | >7.5 | >8.5 | 8.4 | 8.6 | 8.5 | 8.6 |
| 11752 | <i>Fusobacterium varium</i> | 8.3 | 8.3 | 8.4 | 8.3 | 8.3 | 8.4 |

It can be seen that the **Bio-Bag** (Type A) Multi-Plate supported the growth of a variety of organisms from those relatively aerotolerant to strict anaerobes. There was no difference in the size of colonies growing in the **Bio-Bag** (Type A) Multi-Plate and the glove box.

AVAILABILITY

Cat. No. Description

261216 **Bio-Bag™** Environmental Chamber (Type A) Multi-Plate, Ctn. of 50

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