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Diversified Biotech, Inc.

(BBO-556,346))

01.11791EFH

UNITED STATES DISTRICT COURT

DISTRICT OF EASTERN MASSACHUSETTS

DIVERSIFIED BIOTECH, INC. Plaintiff, v. Apogent Technologies Inc., and Nalge Nunc International Defendants.	CIVIL ACTION NO.: COMPLAINT DEMAND FOR JURY TRIAL

Plaintiff, Diversified Biotech, Inc. ("Diversified"), having its principal place of business at 1208 VFW Parkway, Boston, Massachusetts,02132 by way of Complaint against Defendants Apogent Technologies Inc. ("APOGENT"), and Nalge Nunc International ("NALGE NUNC") says:

1. **JURISDICTION AND VENUE**

1.1 This is an action for infringement of patents, 35 U.S.C. §271, arising under the patent laws of the United States and tortious interference with contract and prospective economic relationship. This Court has subject matter jurisdiction over Count I pursuant to 28 U.S.C. §§1331 & 1338, and 17 U.S.C. Section 501, *et seq*. This Court has subject matter jurisdiction over the Massachusetts Counts Two and Three as they are claims joined with substantial and related claims under the patent laws of the United States and, therefore, jurisdiction is conferred by 28 U.S.C. Section 1338(b) and Section 1367. Venue is proper in this jurisdiction under 28 U.S.C. §§1391(b) and (c), 28 U.S.C. §1400(b), and M.G.L. Ch 223A §3.

2. THE PARTIES

2.1 Plaintiff DIVERSIFIED is a Massachusetts corporation headquartered in Boston, Massachusetts at 1208 VFW Parkway. DIVERSIFIED is in the business of licensing technologies from universities and others, overseeing manufacture, documentation, marketing, advertising, and sale of the products produced under said licenses through channels including direct mailing, and networking throughout the scientific community ("Business"). DIVERSIFIED has been in business since August 23, 1984.

2.2 APOGENT is a Wisconsin corporation headquartered at 411 East Wisconsin Ave., 24th Floor, Milwaukee, Wisconsin, 53202, with a

resident agent at CT Corp. Systems, 9 Capitol Street, Concord, NH 03301. This judicial district has personal jurisdiction over NALGE NUNC within the meaning of 28 U.S.C. § 1391(c).

2.3 Upon information and belief, Defendant NALGE NUNC is a Delaware corporation headquartered at C/O Apogent Technologies, 48 Congress Street, Portsmouth, NH. 03809. This judicial district has personal jurisdiction over NALGE NUNC within the meaning of 28 U.S.C. § 1391(c).

2.4 NALGE NUNC is doing business, within the meaning of 28 U.S.C. § 1391(c), through distributors, including, *inter alia*, American Bioanalytical, Inc., of Natick, MA, Scientific Plastics, Inc., Waltham, MA, and Johnson Matthey Catalog Company, Ward Hill, MA.

3. NATURE AND BACKGROUND OF THIS ACTION

3.1. U.S. Patent 5,858,770, (the "770 patent") was issued on January 12, 1999, to Daniel Perlman, of Arlington, Massachusetts, and assigned to Brandeis University, of Waltham, Massachusetts. The '770 patent was duly and legally issued for " Cell Culture Plate with Oxygen and Carbon Dioxide Permeable Waterproof Sealing Membrane", which is attached hereto as Exhibit A.

3.2 Diversified Biotech is the licensee of the '770 patent with rights which are exclusive, worldwide, and of indefinite term.

Brandeis has further authorized DIVERSIFIED to prosecute any and all legal actions which may arise out of infringement of the '770 patent. Diversified is currently marking its patented articles by placing that patent number on the packaging and appropriate labels of such packaging, all in accordance with 35 U.S.C. §287.

3.3 DIVERSIFIED has been selling various products, including "Breath Easy Breathable Membrane" (hereinafter PRODUCTS), based on the '770 patent, since February, 1998.

3.4 On or about January, 2001 DIVERSIFIED became aware of the fact that NALGE NUNC was selling products which appeared to infringe on the '770 patent, and within a few weeks obtained samples of NALGE NUNC's apparently infringing product. Said product was tested forthwith by Dr. Perlman, who verified to a reasonable degree of certainty that DEFENDANT's product did infringe on the '770 patent.

3.5 On or about January, 2001, DIVERSIFIED further became aware that the Defendants have been supplying instructions for use,(Exhibit B) together with said infringing products which were substantially similar to the instructions for use (Exhibit C) provided by DIVERSIFIED with its patented product.

3.6 On or about April 22, 2001 DIVERSIFIED, through its attorney, served a cease and desist letter on APOGENT, a copy of which is attached hereto as Exhibit D.

3.7 APOGENT's attorney, Donald F. Frei, Esq., of Wood, Herron, & Evans, LLP, Cinncinnati, OH 45202, corresponded and conferred with DIVERSIFIED's attorney on several occasions regarding the cease and desist letter, and the matters underlying said letter.

3.8 Despite having acknowledged notice of the infringement by DIVERSIFIED, on information and belief APOGENT continued their unlawful, infringing activities as cited, including infringing sales through its subsidiary, NALGE NUNC, and through its above-mentioned sales agents.

4. FIRST COUNT

PATENT INFRINGEMENT

4.1 DIVERSIFIED repeats and realleges all of the preceding paragraphs of this Complaint as if fully set forth herein.

4.2 On information and belief, APOGENT, together with its subsidiary, NALGE NUNC, has been and is now infringing, contributorily infringing and actively inducing infringement of at least claims 4 et seq. of the '770 patent by making, using, offering to sell and/or selling within this judicial District and elsewhere in the United States, without license or authority from BRANDEIS or DIVERSIFIED, certain products including

"Breathable Sealing Membrane" ("the infringing product") sold by NALGE NUNC International, under the NUNC trademark, and by instructing others how to use this product.

4.3 On information and belief, the infringement of the '770 Patent by APOGENT, NALGE NUNC, and its agents has been willful and deliberate and said Defendants will continue in their infringing activities unless restrained by this Court.

4.4 On information and belief, the Defendants and their agents have profited handsomely through their infringement. At the same time, the Defendants' infringement has caused DIVERSIFIED irreparable harm in its own business.

4.5 On information and belief, the Defendants will continue to profit by their infringing activities, unless these infringing activities are expired.

4.6 DIVERSIFIED has complied with the requirements of 35 U.S.C. Section 287 when applicable.

4.7 DIVERSIFIED has been damaged and will be irreparably injured unless these infringing activities are enjoined.

WHEREFORE, Plaintiff prays:

A) That this Court adjudge that BRANDEIS is the owner of the '770 Patent, and that Plaintiff DIVERSIFIED is the exclusive licensee of said '770 Patent with all rights of recovery under said patent.

B) That this Court adjudge that each claim of the '770 Patent is good and valid in law and that each of the Defendants has infringed said Patent.

C) That a permanent injunction be issued enjoining all the Defendants and those in privity with the Defendants from further infringement, contributory infringement and actively inducing infringement of said '770 Patent;

D) That an accounting be had for the damages to DIVERSIFIED, separately, arising out of each of the Defendants' infringing activities, together with interest and costs, and that such damages be awarded to DIVERSIFIED;

E) That the Defendants' infringement be adjudged willful and that the damages to DIVERSIFIED be increased to three times the amount found or assessed pursuant to 35 U.S.C. Section 284;

F) That this be adjudged an exceptional case and that DIVERSIFIED be awarded its attorney fees in this action pursuant to 35 U.S.C. Section 285; and

G) That DIVERSIFIED be awarded such other and further relief as the Court may deem just and equitable.

5. SECOND COUNT

INTENTIONAL INTERFERENCE WITH PROSPECTIVE ECONOMIC RELATIONSHIP

5.1 DIVERSIFIED repeats and realleges all of the preceding paragraphs of this Complaint as if fully set forth herein.

disrupting Plaintiff's existing and prospective economic advantage as described herein.

5.7 Plaintiff is informed and believes and alleges thereon that Defendants have infringed Plaintiff's '770 Patent in soliciting Plaintiff's customers, and other customers who were considering doing business with Plaintiff, with the intent of interfering with the economic advantage Plaintiff enjoyed and anticipated enjoying with these customers.

5.8 Plaintiff is informed and believes and thereupon alleges that Defendants intentionally interfered with such economic advantage for the purpose of causing Plaintiff harm and injury, and acted with malice, oppression and the intent to defraud. Plaintiff is, therefore, entitled to punitive damages in an amount sufficient to punish Defendants. Such wrongful acts were performed by officers of the corporate Defendants and/or were ratified by the corporate Defendants.

5.9 Plaintiff has no adequate remedy at law to fully protect itself from Defendants' violations of Plaintiff's rights, which violations are continuing, and, unless restrained by this Court, will continue, and which are subjecting Plaintiff to immediate and irreparable harm to Plaintiff's reputation and the ability to stay in business. Accordingly, Plaintiff seeks injunctive

product in their possession or control, whether paid for or otherwise, at the conclusion of this action.

E. That Defendants be permanently enjoined from assisting, aiding or abetting any other person or business entity in engaging in or performing any of the activities enumerated in paragraphs A and B above.

F. That Defendants issue written notice to all of their customers, including those to whom it has sold or offered for sale the infringing product, which states that said breathable membranes are not endorsed, approved, manufactured or authorized by Plaintiff.

G. That monetary damages be awarded to Plaintiff in an amount to be fixed by the Court, including all of Defendants' profits or gains resulting from their interference with Plaintiff's prospective business relationships, or awarding Plaintiff its damages, whichever is greater, said amount to be trebled and exemplary damages to be awarded in view of Defendants' intentional acts.

H. Awarding to Plaintiff its attorneys' fees due to the exceptional nature of this case, and all of its costs and expenses of litigation.

I. For such other and further relief as this Court may deem just and proper.

6. THIRD COUNT

INTENTIONAL INTERFERENCE WITH CONTRACTUAL RELATIONS

6.1. DIVERSIFIED repeats and realleges all of the preceding paragraphs of this Complaint as if fully set forth herein.

6.2. At the time Defendants committed the actions alleged above, Plaintiff enjoyed existing contractual relations with its main customers. Plaintiff is informed and believes and alleges thereon that Defendants infringed Plaintiff's licensed Patent '770, and used such intellectual property in soliciting Plaintiff's customers with the intent to interfere with the existing contractual relationships Plaintiff enjoyed with its main customer base and its other customers.

6.3. As a proximate result of the Defendants' wrongful acts as described above, Plaintiff has been damaged in an amount to be proven at trial.

6.4. Plaintiff is informed and believes and thereupon alleges that Defendants intentionally interfered with such contractual relationships for the purpose of causing Plaintiff harm and injury, and acted with malice, oppression and the intent to defraud Plaintiff. Such acts were performed by officers of the corporate Defendants and/or were ratified by the corporate Defendants.

6.5. Plaintiff has no adequate remedy at law to fully protect itself from Defendants' violations of Plaintiff's rights, which violations are continuing, and, unless restrained by this Court, will continue, and which are subjecting Plaintiff to irreparable harm to Plaintiff's reputation and the ability to stay in business. Accordingly, Plaintiff seeks injunctive relief prohibiting Defendants from engaging in such wrongful activities.

WHEREFORE, Plaintiff prays:

A. That Defendants, their directors, officers, agents, servants, employees, related companies, parent companies, subsidiaries, licensees, assigns, and all parties in privity with Defendant, be permanently enjoined from intentionally interfering with the contractual relationships between Plaintiff and its customers in any manner, including, but not limited to, selling or offering for sale to any of Plaintiff's customers any products that are competitive to Plaintiff's products and which contain products which infringe the '770 patent.

B. That Defendants file with this Court and serve upon Plaintiff within thirty (30) days after service of the injunction demanded above, a report, in writing, under oath, setting forth in detail the manner and form in which it has complied with the injunction.

C. That Defendants be required to deliver up to be impounded during the pendency of this action, all infringing exemplars of the Defendants' infringing product in their possession or control.

D. That Defendants be permanently enjoined from assisting, aiding or abetting any other person or business entity in engaging in or performing any of the activities enumerated in paragraphs A and B above.

E. That Defendants issue written notice to all of their customers, including those to whom it has sold or offered for sale the infringing product, which states that said products are not endorsed, approved, manufactured or authorized by Plaintiff.

F. That monetary damages be awarded to Plaintiff in an amount to be fixed by the Court, gains resulting from their interference with Plaintiff's contractual business relationships, or awarding Plaintiff its damages, whichever is greater, said amount to be trebled and exemplary damages to be awarded in view of Defendants' intentional acts of infringement.

G. Awarding to Plaintiff its attorneys' fees due to the exceptional nature of this case, and all of its costs and expenses of litigation.

H. For such other and further relief as this Court may deem just and proper.

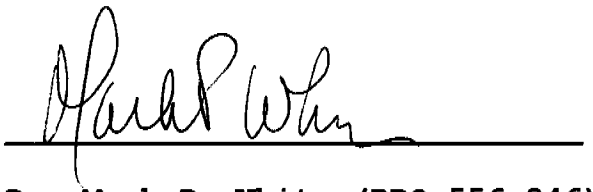
JURY DEMAND

Plaintiff hereby demands a trial by jury as to all issues so triable.

Mark P. White

Attorney for Plaintiff,

Diversified Biotech, Inc.



By: Mark P. White (BBO 556,246)

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Exhibit A

US005858770A

United States Patent [19]

Perlman

[11] Patent Number: 5,858,770

[45] Date of Patent: Jan. 12, 1999

[54] **CELL CULTURE PLATE WITH OXYGEN AND CARBON DIOXIDE-PERMEABLE WATERPROOF SEALING MEMBRANE**

[75] Inventor: **Daniel Perlman**, Arlington, Mass.

[73] Assignee: **Brandeis University**, Waltham, Mass.

[21] Appl. No.: **940,422**

[22] Filed: **Sep. 30, 1997**

[51] **Int. Cl.**[6] **C12M 3/00**

[52] **U.S. Cl.** **435/305.3**; 435/305.4; 435/297.5

[58] **Field of Search** 435/325, 374, 435/243, 260, 297.1, 297.5, 305.1, 305.3, 305.4, 307.1; 422/102

[56] **References Cited**

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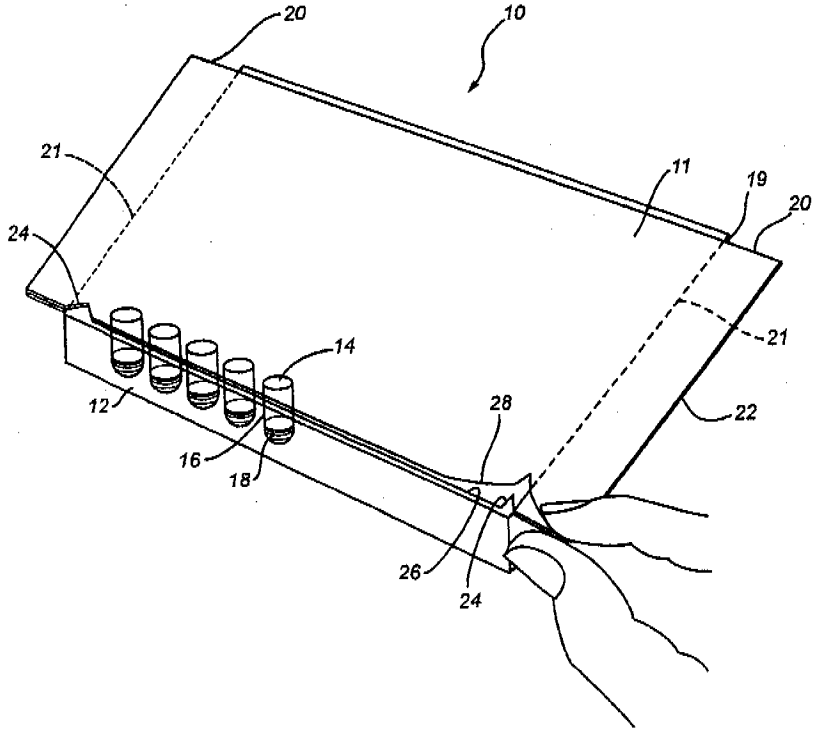
Primary Examiner—David A. Redding

Attorney, Agent, or Firm—Lyon & Lyon LLP

[57] **ABSTRACT**

A cell culture plate which contains cells incubated in a liquid medium held in sample wells formed and arranged over the surface of the plate, is covered and sealed with a waterproof adhesive sealing membrane which excludes microbial contaminants, but which is functionally permeable to oxygen and carbon dioxide gases. The membrane allows cellular respiration to continue, with the transmission rate of oxygen and carbon dioxide through the membrane being essentially uniform from well to well over the surface of the plate.

11 Claims, 1 Drawing Sheet



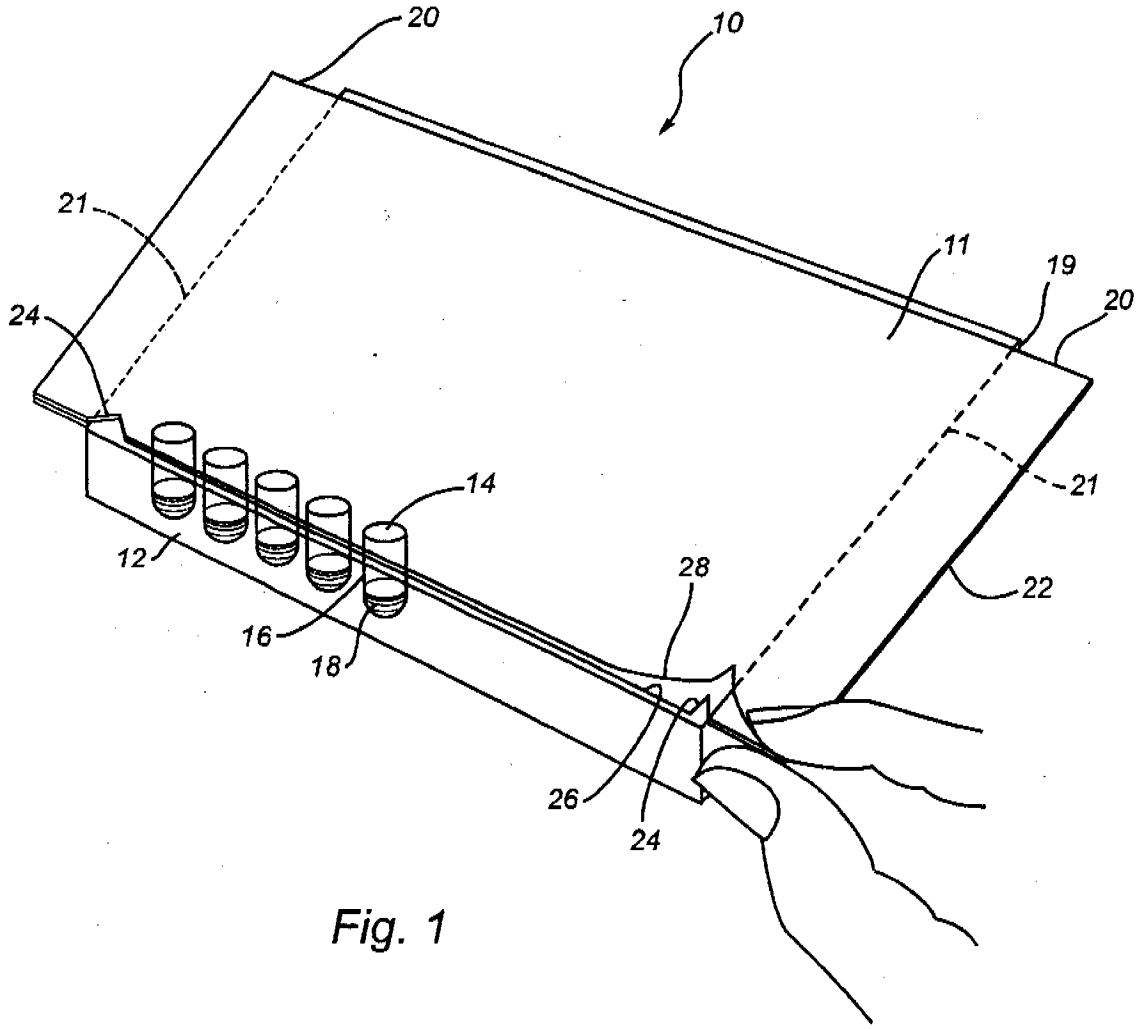


Fig. 1

CELL CULTURE PLATE WITH OXYGEN AND CARBON DIOXIDE-PERMEABLE WATERPROOF SEALING MEMBRANE

BACKGROUND OF THE INVENTION

This invention relates to a cover for a multi-sample container, of a variety known as a "cell culture plate" or a "microtiter plate" for culturing and manipulating living cells. Specifically, the invention concerns cell culture plates in which the plate is sealed with an oxygen and carbon dioxide-permeable waterproof sealing membrane.

Cell culture plates including the so-called "multiwell microliter plates" (herein collectively abbreviated and termed "culture plates" or "plates") are shallow plastic vessels with multiple compartments, i.e., sample wells, which are used for holding growth medium for culturing cells. Culture plates are currently manufactured from polystyrene, polyethylene, polypropylene, polycarbonate, and polyvinyl thermoplastic resins using conventional injection-molding or thermoforming methods. The most common present day culture plates measure approximately 3 inches by 5 inches and are physically described, depicted and commercially available in most scientific supply catalogs (see, for example, Nunc Products 1996 catalog, Nalge Nunc International, Naperville Ill.). Culture plates typically contain between 4 and 384 sample wells per plate. Depending upon the size of wells in a plate, the liquid capacity of each well ranges from approximately 100 microliters to 2 milliliters.

Living cells contained in the sample wells of culture plates can be tested or assayed for physical, chemical and biological properties during or after cell growth using biochemical and immunochemical techniques and reagents. Growth and/or reliable testing of cells in the multiple wells of such plates depends upon the plates being free of cytotoxic substances, ongoing sterility being maintained within the wells of the plates, and uniform dependable oxygen and carbon dioxide exchange into and out of the wells. For maintaining sterility, either of two commercially available covers may be used on the plates during or after cell growth. The covers protect not only living cells within the sample wells, but also their metabolites and any associated sterile reagents added to the wells. The first type of cover is principally used during cell growth, and is a loose-fitting molded plastic lid which overhangs the plate to exclude particulate contaminants (such as bacterial contaminants), while allowing exchange of gases for support of cellular respiration and cell growth. The second type of cover is an essentially gas-impermeable adhesive polyester, polypropylene, or polyethylene plastic sealing film (e.g., Sealplate™, manufactured by Excel Scientific, Inc., Wrightwood, Calif.). Such sealing films function to seal each sample well, and prevent well to well contamination and sample evaporation, while being chemically inert so as to allow chemical processing steps to be carried out in the plate. Since this second type of cover is gas-impermeable, it is only applied to the plate following cell growth. It is most useful for freezer storage, preservation, and the chemical processing of cells and other micro-samples.

SUMMARY OF THE INVENTION

In general, the present invention is directed toward improving the multiwell cell culture plate, by providing a gas-permeable, leak-proof, and adhesive membrane for sealing such plates. Culture plates generally have a multiplicity of compartments, i.e., four or more sample wells, which are

used for holding and culturing living cells. Because an adhesive covering and sealing membrane has been found which is oxygen and carbon dioxide-permeable, i.e. "breathable", as well as being waterproof and non-cytotoxic, living cells of either eukaryotic or procaryotic origin, can grow and thrive within a sealed leak-proof culture plate. A variety of cell types including mammalian and bacterial cells have been shown to retain normal viability and growth rates after they and their culture medium contact the membrane. An example of a useful membrane is one that is generally thin and composed principally of polyurethane polymers, e.g., one that is currently used as a moisture-permeable surgical dressing. It does not peel off any of culture plates tested, nor allow any aqueous liquids to leak through either when inverted with liquid culture medium held in the sample wells, or when placed in an incubator at 37° C. and 100% relative humidity. The addition of an oxygen and carbon dioxide-permeable sealing membrane provides benefits and allows cell manipulation procedures in a culture plate to be routinely carried out, which were not previously practical. Procedures which previously required chemical assay steps with living cells, required the use, (albeit brief so as not to kill the cells) of gas-impermeable sealing membranes (sealed to permit liquid shaking, inversion of the plate, etc.). Such procedures can now be carried out easily and over longer time intervals because breathable sealing membranes allow respiration, cell viability and cell growth to be maintained in the shaken and inverted leak-proof culture plates. In fact, many cellular-based assays depend upon continuing respiration for accuracy and reproducibility of the assays, and an extended period of ongoing cellular metabolism may be required for cells held in such plates. Furthermore, Applicant has discovered that while prior art hard plastic covers allow rapid gas exchange around their perimeters, they allow a diminishing rate of gas exchange toward the center of the plate. This limitation is overcome by the membranes of the present invention which assures uniformity of gas exchange and thus cellular respiration from well to well and sample to sample across the entire plate. This uniformity is important for experimental accuracy and valid comparisons among different cell samples held in different wells within a plate.

Considering the prior covers more specifically (see above), cover type (i) is impermeable and fabricated of rigid plastic which, as a loose-fitting cover, allows gas exchange (via leakage and exchange of oxygen and carbon dioxide around the perimeter of the plate) and cell growth. On the other hand for cover type (ii), the prior cover includes sterile impermeable adhesive sealing films which create a waterproof and microbe-impermeable seal over and around each well. Significant limitations exist in the use of both of these two covers. In fact, there are many analytical procedures which cannot be carried out efficiently in culture plates due to limitations in the utility of these covers identified by Applicant. More specifically, the use of a plastic cover, (i), which allows general cell growth to continue, is not ideal because Applicant has discovered that gas exchange is non-uniform from sample well to sample well across the culture plate. Wells adjacent the corners and edges of the plate experience a higher rate of gas exchange than the more distant wells nearer the center of the plate. Consequently, cellular-based assays which depend upon uniform cellular respiration and oxygen tension among the sample wells, may suffer from variable or unreproducible results from well to well across the plate. On the other hand, the use of the prior art sealing film, (ii), provides only a waterproof barrier useful for storing and freezing cells, and useful for chemical,

biochemical and immunological assay of cells. For example, chemical and biochemical reagents can be placed into sample wells containing cells, and the sealing film allows the reagents in the culture plate to be shaken without leakage or well to well cross-contamination. However, such a sealing film does not allow cellular respiration to continue. Therefore, the sealing film does not allow a prolonged or continuous incubation of living (respiring) cells with reagents. In fact, the combined or sequential use of the covers (i) and (ii) does not in any practical sense allow continuous incubation of living cells in a culture plate, with simultaneous inversion and/or shaking, for example, to maintain a mixed suspension of living cells and reagents.

Applicant has discovered that in the technology area of human wound care dressings, the sterile moisture-permeable, flexible membranes which have become commercially available for application to the skin (following surgery, wound injury or burn injury) are also highly permeable to oxygen and carbon dioxide. These membranes are typically thin (approximately 1 mil, i.e., 0.001 inch thick) and fabricated from a moisture-permeable copolymer such as polyester-polyurethane or polyether-polyurethane. Moisture permeation through the membrane is important for its wound care use, so that the membrane does not trap liquid perspiration (from sweat glands in the skin). While these wound dressing membranes are thin, they do not allow either microorganisms or viruses to pass through. Consequently, when applied and adhered to a culture plate as described in the present invention, microbial contaminants are likewise excluded from the sample wells of the plate. Those in the art will recognize that any adhesive and non-cytotoxic membrane that is impermeable to microorganisms such as bacteria, mycoplasma and even viruses is useful in this invention so long as sufficient oxygen and carbon dioxide-permeability exists through the membrane. The amount of gas permeability necessary will depend on the experiment to be conducted, but will generally be comparable to the membranes exemplified herein. Standard tests can be used to determine adequate permeability for the purposes of this invention, as well as the other preferred features of the membranes noted below.

DEFINITIONS

For the purposes of this invention, and to aid in the selection of appropriate sealing membrane materials and related component materials for covering and sealing culture plates, it is important to define a number of technical and generally descriptive terms used in the text, and in the claims. The term "a waterproof adhesive sealing membrane" refers to a thin sheet material, i.e., a manufactured film, which blocks any measurable permeation by liquid water and aqueous solutions, and which is coated on its lower surface with a pressure-sensitive adhesive. Furthermore, neither the film nor the adhesive can dissolve in water, nor leach any measurable quantity of substances harmful to the growth of cultured eukaryotic and prokaryotic cells during exposure to aqueous solutions, nutrient growth medium, and the like. In the context of the properties of the membrane, the term "excludes microbial contaminants: refers to the ability of the membrane when attached to a cell culture plate, to prevent penetration of bacteria and viruses which are placed on the outside, i.e., upper surface of the membrane. The membranes described in the present invention, and previously used a wound care dressings, have been thoroughly tested for their ability to exclude a wide range of bacteria and viruses. The term "permeable" in the context of membrane permeability to oxygen and carbon dioxide is defined

in the "Example" presented below. The term "cellular respiration" refers to the metabolic processes in a living cell which are dependent upon availability of oxygen to the cell, to generate energy for cellular growth and other functions, and the removal and balancing of carbon dioxide in the immediate environment of the cell for maintaining normal pH within the cell. The term "sufficiently permeable" in the context of oxygen and carbon dioxide permeability of a membrane, is a relative term referring to the functional ability of a membrane to allow essentially the same rate of cell growth and rate of pH equilibration (in the liquid medium surrounding cells in the sample wells of the culture plate), upon comparison with identical cells grown in a culture plate covered with a conventional loose-fitting hard plastic cover. The term "essentially uniform transmission rate" refers to the absence of variability in the rate of membrane permeation by oxygen and carbon dioxide as observed across the length and width of a given membrane covering a cell culture dish (see "Example" presented below). It is also useful to define several liquid media used for incubating, washing and treating cells. Thus, "nutrient growth medium" is a liquid which is used to feed living cells and support their growth, and contains vitamins, essential nutrients, salts and the like. "Incubation buffer medium" is a liquid which helps sustain cellular viability and electrolyte balance in a cell, without containing the nutrients to support cellular growth and proliferation. "Buffered enzyme medium" is equivalent to an "incubation buffer medium", bu also containing one or more enzymes which are included to alter the cells or their metabolites. Thus trypsin, the digestive enzyme, may be added to an incubation buffer medium to help disaggregate aggregated or cohered eukaryotic cells. "Chemical, biochemical or immunochemical assay medium" is equivalent to an "incubation buffer medium", but also contains one or more chemical, biochemical or immunochemical reagents which react with cells and/or metabolites produced by cells to help identify individual cells or groups of cells, and/or to help measure the level of a metabolite (e.g., a small molecule such as a sugar or amino acid, or a macromolecule metabolites such as an antibody) produced by such cell(s), or to measure and/or detect some other biochemical marker being studied in the cell(s). An immunochemical reagent can include, for example, a specific antigen used to detect the presence of an antibody or antibodies produced by a cell or group of cells, or alternatively, an antibody reagent used to complex with, and detect a desired antigen. The term "facestock material", refers to a substrate film whose lower surface, in the present invention, is coated with an adhesive. The substrate film or facestock is typically formed from an extruded or cast polymer or copolymer sheet material. Additional sheets or films may be added to the upper surface of the facestock, such as a removable carrier film, to provide additional rigidity to thin facestocks, to aid in the manual handling and aligning of the membrane on the culture plate. Furthermore, a removable release sheet may be attached to the lower surface or adhesive coating on the facestock to protect the adhesive from contaminating substances such as microbes, prior to use. The term "removable", in the context of a sheet or film is defined as manually peelable. The term "non-cytotoxic" refers to the cell compatibility of a material (such as the facestock material and pressure-sensitive adhesive used herein) which allows that material to remain in contact with living cells and the liquid medium being used to bathe and/or incubate these cells, without the metabolism, growth rate, and viability of the cells being adversely affected. The term "sterile" or "sterility" as used herein, refers to the

absence of any active microbes, i.e., any viable cellular contaminants, mycoplasma, and/or infectious viruses and phages. By definition, when a sealing membrane is adhesively attached to a culture plate, and is capable of "excluding" microbial contaminants, it can "maintain the sterility" in the sample wells of the plate. That is, the pore size of the membrane is too small to allow passage of microbes. Likewise, a removable release paper (such as a conventional 50 pound Kraft release paper) is typically used to cover and protect the lower surface of the membrane and the adhesive coating material thereon, and serves to maintain the sterility of this surface and coating prior to use.

In a first aspect, the present invention features a cell culture plate containing cells which are incubated in a liquid medium contained in sample wells formed and arranged over the surface of the plate. The plate is covered and sealed with a waterproof adhesive sealing membrane which excludes microbial contaminants, but is functionally permeable (as defined above) to oxygen and carbon dioxide gases, allowing cellular respiration to continue, with the transmission rate of these gases through this sealing membrane being essentially uniform from well to well over the surface of the plate.

In preferred embodiments, the liquid medium used for incubation of the cells in these culture plates is selected from the group consisting of nutrient growth medium, incubation buffer medium, buffered enzyme medium, chemical assay medium, biochemical assay medium, and immunochemical assay medium. For example, growth media having defined compositions are well known in the art such as Eagles and Dulbeccos media for eukaryotic cells, and Luria's broth and M9minimal media for bacterial cells. Similarly, cell incubation buffers and buffered enzyme solutions of defined compositions are known, such as phosphate buffered saline (PBS) and PBS with trypsin enzyme for disaggregating and resuspending cells. Likewise, numerous buffered chemical, biochemical and immunological solutions have been defined and used in these culture plates. In other preferred embodiments, the adhesive sealing membrane includes a facestock material selected from the group consisting of polyester-polyurethane copolymer film and polyether-polyurethane copolymer film. These copolymers and thin films (such as 0.001 inch thick films) formed with these copolymers are known in the field of surgery and wound care because these films are substantially moisture-permeable. In a particularly preferred embodiment, the facestock of these films has a thickness ranging between 0.0005 and 0.002 inches. This thickness range not only allows water vapor permeation, but also allows adequate diffusion of O_2 and CO_2 to support cell respiration and growth in the culture plates.

In still another embodiment, the bottom, i.e., the lower surface, of the sealing membrane is coated with a non-cytotoxic pressure-sensitive adhesive material which adheres the sealing membrane to said culture plate. In a more preferred embodiment, the adhesive is a non-cytotoxic pressure-sensitive acrylic adhesive material.

In a second related aspect, the present invention features a kit which includes a culture plate for culturing living cells which are incubated in a liquid medium placed in sample wells formed and arranged over the surface of the culture plate, and a sterile sheet assembly which includes a sized portion of the waterproof sealing membrane described above in the first aspect of the invention.

In preferred embodiments, the sized portion of membrane includes a first lower surface which is coated with a non-cytotoxic pressure-sensitive adhesive material for attaching the membrane to the culture plate; the pressure-sensitive adhesive material is covered and protected by a removable release paper sheet which serves to maintain the sterility of the first lower surface and this pressure-sensitive adhesive material; the sterile sized portion of membrane has a second upper surface which is at least partially covered by a removable upper carrier sheet which aids in handling and aligning the thin and floppy membrane during its attachment to the culture plate; and the removable upper carrier sheet is selected from the group consisting of a peelable transparent plastic sheet, and a peelable release paper frame configured and arranged around the perimeter portion of the membrane.

In a third related aspect, the invention features a sterile sheet assembly which includes a sized portion of waterproof adhesive sealing membrane which is functionally permeable (as defined above) to oxygen and carbon dioxide gases. The membrane includes a first lower surface which is coated with a non-cytotoxic pressure-sensitive adhesive material for attaching the membrane to a culture plate. The adhesive material is protected by a removable release paper sheet which serves to maintain the sterility of this first lower surface and adhesive material. The release paper sheet is cleaved, i.e., fully scored or cut through its thickness and across its width at two locations, with each of two cleavages positioned not less than 0.2 inches, and not more than 1.5 inches from each of the two ends of the assembly. These two cleavages define the boundaries of two release paper-covered end tabs for manually handling, aligning and adhesively attaching the membrane to the culture plate after the remainder of the release paper sheet has been stripped away from the adhesive surface.

In preferred aspects, the sterile sheet assembly is provided which includes a sized portion of waterproof adhesive sealing membrane which is functionally permeable to oxygen and carbon dioxide gases. The membrane includes a first lower surface which is coated with a non-cytotoxic pressure-sensitive adhesive material for attaching the membrane to a culture plate. The adhesive material is protected by a removable release paper sheet which serves to maintain the sterility of the first lower surface and the adhesive material. The membrane includes a second upper surface which is at least partially covered by a removable upper carrier sheet which provides a degree of rigidity for manually handling the thin and floppy membrane during its alignment and attachment to the culture plate. The assembly includes at least one edge-notch along its length. This notch is cut through all of the layers of the sheet assembly, and is positioned, i.e., is notched, not less than 0.2 inches, and not more than 1.5 inches from the end of the assembly. The notch allows convenient shearing force-separation, peeling and removal of the upper carrier sheet after the membrane has been adhered to the culture plate. Removal of this upper carrier sheet is necessary before functional permeability to O_2 and CO_2 can be achieved by the membrane. In a preferred embodiment of this aspect, the edge-notch in the sheet assembly extends no more than 0.25 inches inward from the edge of this sheet assembly, and the geometric shape of this edge-notch is selected from the group consisting of a V-notch, a cove cut (U-shaped notch), and a straight slit.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments, and from the claims.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a perspective view in partial section showing a gas-permeable membrane attached to a cell culture plate,

and an upper carrier sheet being separated for removal from this membrane.

APPARATUS

Referring to FIG. 1, covered cell culture plate **10** is assembled from a sterile sheet assembly **11** and a cell culture plate **12**. Sheet assembly **11** consists of three layers, an upper layer which serves as a carrier sheet **28**, a middle layer which functions as the breathable adhesive sealing membrane **26**, and a lower layer which functions as a release paper sheet **22** to protect the adhesive coating. The culture plate **12** contains a rectangular matrix of sample wells, e.g., ninety-six wells, **16** (only five of which are shown) whose 0.25 inch diameter openings **14** lie on the surface, or just above the surface of plate **12**. These wells **16** typically contain buffered liquid **18**, e.g., cell growth medium and living cells which require free exchange of oxygen and carbon dioxide gases for respiration. Sheet assembly **11** is elongated with end portions **20** which overhang the end **19** of the cell culture plate to facilitate manual handling and aligning the sheet assembly on the culture plate **12**. Before adhering sheet assembly **11** to culture plate **12**, a release paper sheet **22** is peeled from the underside of assembly **11** except on end portions **20** of the assembly where parallel cleavages, i.e., scoring cuts **21** across the underside of assembly **11**, and through release paper sheet **22** allow the paper to remain adhered to the underside of end portions **20**. The sterile adhesive underside of sheet assembly **11** is aligned and uniformly adhered to the top of culture plate **12** using a clean and compliant rubber faced ink brayer to establish uniform surface adhesion. Next, one of the corners of the end portion **20** of sheet assembly **11** is pulled gently to the side using ones fingers as shown. During this pulling, notch **24** (previously cut through all of the layers of the sheet assembly **11**) serves to focus sheering forces between the two remaining layers (membrane **26** and carrier sheet **28**) of sheet assembly **11**, allowing a physical separation, peeling, and removal of upper carrier sheet **28** (transparent polyethylene which is not very stretchable) from a breathable waterproof adhesive sealing membrane **26** (polyether or polyester-polyurethane which is very stretchable) which remains on the cell culture plate.

EXAMPLE

While the moisture permeability of a membrane may be important for a wound dressing, Applicant was concerned with the oxygen (O_2) and carbon dioxide (CO_2) exchange rates across a given membrane, because these rates must be adequate to sustain normal respiration of living cells held in culture plates. Accordingly, a two part test method was developed which can be used to determine whether a particular membrane is suitable and useful for the purposes of the present invention. As described below, CO_2 permeability was monitored by rate of color change of a pH indicator in the nutrient growth medium in culture plate, while O_2 permeability and lack of cytotoxicity were coordinately monitored by cell growth rate (versus identical "control" cells grown in a culture plate with a conventional hard plastic cover). Two wound dressings were initially tested, one a 1 mil thick polyether polyurethane membrane known as Tegaderm™ manufactured by 3M Health Care, St. Paul, Minn., and the other, a similar 1 mil thick polyurethane membrane known as S/C urethane film product #8167-08 manufactured by Medco Coated Products, Bedford, Ohio. The ability of these membranes to allow exchange of CO_2 was monitored in a cell culture "gas incubator" in which 5% CO_2-95% air is fed to the cells. Three identical culture plates (with 96 wells), all containing Eagles cell growth medium (with phenol red pH-indicator for monitoring incoming dissolved carbon dioxide) were used in this experiment. One plate was covered with a standard commercial rigid plastic lid, while the other two were adhesively "sealed" with the breathable membranes of the present invention. When placed in a gas incubator, as carbon dioxide diffuses into growth medium held in a standard culture plate covered with a conventional plastic lid (i), the color of the pH indicator changes from dark pink to reddish-orange as the pH increases. Observations were made on the pH indicator color change in individual sample wells, comparing the conventionally covered plate with the membrane-sealed plates, as a function of time in the incubator. Observations showed that CO_2 exchange occurred in all plates. However, CO_2 transfer (color change) was slowest in the center area of the conventionally covered plate. Color change was more rapid in the perimeter area of this conventionally covered plate, and comparable to rate of color change in the membrane-sealed plates.

For the purposes of the present invention, the approximate equality or parity between the CO_2 transfer rate (measured by observed rate of pH indicator color change) in conventional lid-covered culture plates, and the two breathable membrane-covered plates defines the breathable membranes used in the present invention as functionally CO_2-permeable. Similarly, comparable cell growth rates observed for hybridoma cells cultured under the conventional lid and the two membrane coverings, defined the breathable membranes as being functionally O_2-permeable.

Of significant benefit and utility is the elimination of well to well experimental variability due to variable gas exchange rates with cellular-based assays. The polyurethane membrane-sealed plates showed complete uniformly of indicator color change from well to well across the plates. Moreover, the waterproof acrylic adhesives present on both the 3M and Medco polyurethane membranes, and used to adhere them to the culture plates were shown to be non-cytotoxic. This conclusion was based upon culturing varying dilutions of mouse-human hybridoma cells initially suspended in growth medium on the adhesive underside of the membranes (inverting the culture plates, with the adhesive surface facing downward). All cell dilutions grew up to normal confluent cell densities indicating that the membranes and adhesives have no detectable degree of cytotoxicity.

Besides providing uniformity of gas exchange across the culture plates, the breathable adhesive sealing membranes of the present invention allow new, simplified, and more rapid cellular-based assays and procedures to be carried out in culture plates using these membranes. In addition to the two membrane materials described and tested above, a number of other membrane films typically used as moisture-permeable surgical and skin-protective dressings are commercially available and can function as O_2 and CO_2-breathable membranes which also exclude microorganisms and viruses, including the transparent Bioclusive™ membrane manufactured by Johnson and Johnson Medical, Inc. (Arlington, Tex.).

To minimize the chance of liquid leakage around sample wells across the culture plate, it has been found that following manual alignment and initial adhesive contact between the breathable membrane and the culture plate, a rubber-faced brayer ink-type roller can be used to apply uniform pressure over the surface of the membrane and eliminate any discontinuities or air bubble voids which could lead to such leakage. One such suitable roller is a firm rubber-faced

brayer, model #24, lightweight, approximately four inches wide and one inch in diameter, available from the Testrite Instrument Company, Newark, N.J.

I claim:

1. A cell culture plate comprising one or more cells held within a liquid medium contained in a plurality of wells formed and arranged within said plate, wherein said wells are covered and sealed with a waterproof adhesive sealing membrane which excludes microbial contaminants, and is permeable to oxygen and carbon dioxide gases, and wherein a first lower surface of said sealing membrane is coated with a non-cytotoxic pressure-sensitive adhesive material which adheres said sealing membrane to said culture plate.

2. The plate of claim 1, wherein said membrane is sufficiently permeable to said gases to allow cellular respiration to continue.

3. The plate of claim 1, wherein said membrane is designed to allow the transmission rate of said gases through said membrane to be essentially uniform from well to well within said plate.

4. The culture plate of claim 1, wherein said liquid medium is selected from the group consisting of nutrient growth medium, incubation buffer medium, buffered enzyme medium, chemical assay medium, biochemical assay medium, and immunochemical assay medium.

5. The culture plate of claim 1, wherein said sealing membrane comprises a facestock material selected from the group consisting of polyester-polyurethane copolymer film and polyether-polyurethane copolymer film.

6. The culture plate of claim 1, wherein said sealing membrane comprises a facestock selected from the group consisting of polyester-polyurethane copolymer film and polyether-polyurethane copolymer film, wherein said facestock has a thickness ranging between 0.0005 and 0.002 inches.

7. The culture plate of claim 1, wherein said pressure-sensitive adhesive material is a non-cytotoxic pressure-sensitive acrylic adhesive material.

8. A kit comprising a culture plate comprising sample wells formed and arranged within said culture plate, and a sterile sheet assembly comprising a sized portion of the waterproof sealing membrane which excludes microbial contaminants, and is permeable to oxygen and carbon dioxide gases, and which comprises a first lower surface which is coated with a non-cytotoxic pressure-sensitive adhesive material for attaching said membrane to said culture plate.

9. The kit of claim 8, wherein said pressure-sensitive adhesive material is covered and protected by a removable release paper sheet which serves to maintain the sterility of said first lower surface and said pressure-sensitive adhesive material.

10. The kit of claim 8, wherein said pre-sterilized sized portion of membrane comprises a second upper surface which is at least partially covered by a removable upper carrier sheet which aids in the manual handling of said membrane during its alignment and attachment to said culture plate.

11. The kit of claim 10, wherein said removable upper carrier sheet is selected from the group consisting of a peelable transparent plastic sheet, and a peelable release paper frame configured and arranged around the perimeter portion of said membrane.

* * * * *

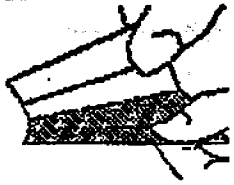
163340

Breathable Sealing Membrane

featuring 3M™ Adhesive Technology

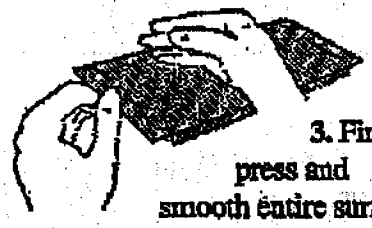
80 mm x 124 mm, Sterile

How To Apply NUNC™ Breathable Sealing Membrane to a MicroWell® Plate



1. Remove from pouch and separate white papers. Breathable sealing membrane, with adhesive surface exposed, remains attached to shorter white carrier paper.

2. Position white carrier paper with membrane adhesive surface facing towards plate. Center and lower onto plate.



3. Firmly press and smooth entire surface of white carrier paper with applicator, roller or hand, to ensure uniform adhesion of membrane to plate.

4. To remove carrier paper, crease one of the short edges to conform to the plate edge, then lift. Membrane is now affixed to plate.

Nalge Nunc International

NUNC™ Brand Products

Nalge Nunc International Corp., Naperville, IL USA
Nunc A/S, Roskilde, Denmark

Caution: The packaging of this product contains natural rubber latex which may cause allergic reactions.

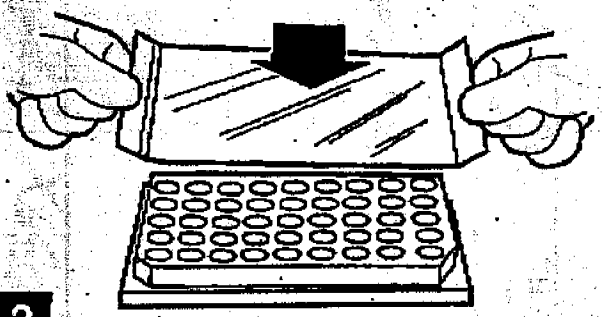
2300-10 AL

Exhibit C

Breathe-Easy™

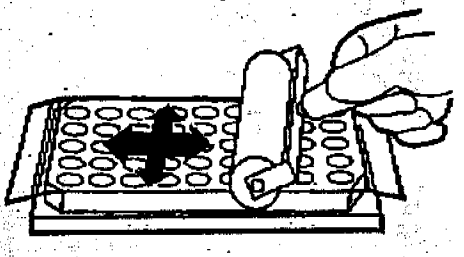
Step 1.

Peel away paper backing and apply to plate



Step 2.

Roll brayer several times in all directions to ensure uniform adhesion to the plate.



Step 3.

Top protective layer can be easily separated from the membrane by inserting a finger between the top protective layer at the longer end tab and pulling up gently.

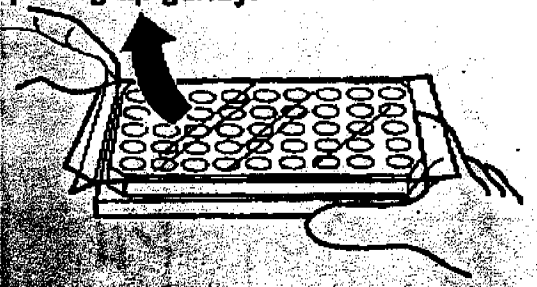


EXHIBIT D

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April 22, 2001

BY CERTIFIED MAIL NO: P 285 813 477
RETURN RECEIPT REQUESTED

Apogent Technologies, Inc.
411 East Wisconsin Ave.
24th Floor
Milwaukee, WI 53202
ATTN: President or CEO

Dear Sir or Madam:

I represent Diversified Biotech, Inc. (hereinafter DIVERSIFIED), of 1208 VFW Parkway Boston, Massachusetts 02132.

Diversified Biotech is the licensee of U.S. Patent 5,858,770, issued on January 12, 1999, to Daniel Perlman, of Arlington, Massachusetts, and assigned to Brandeis University, of Waltham, Massachusetts. The "770" patent was duly and legally issued for " Cell Culture Plate with Oxygen and Carbon Dioxide Permeable Waterproof Sealing Membrane", which is attached hereto as Exhibit A.

DIVERSIFIED's rights in the "770" patent are exclusive, worldwide, and of indefinite term. Brandeis has further authorized DIVERSIFIED to prosecute any and all legal actions which may arise out of infringement of the "770" patent. DIVERSIFIED is currently marking its patented articles, by placing that patent number on the packaging and appropriate labels in such packaging, all in accordance with 35 U.S.C. §287. DIVERSIFIED has been selling various products, including "Breath Easy Breathable Membrane" (hereinafter PRODUCTS), based on the "770" patent, since February, 1998..

It has come to our attention that Apogent Technologies, Inc. (hereinafter Apogent), its divisions, joint ventures, and affiliate companies, is selling at least one product which infringes on one or more claims of the "770" patent, including a "Breathable Sealing Membrane " sold by Nalge Nunc International, under the NUNC trademark. On information and belief, the "Breathable Sealing Membranes" are currently being sold throughout the United States and abroad.

The sale of the "Breathable Sealing Membrane", together with a "plurality of wells formed and arranged within (said) plate", claim 1, constitutes an infringement of the "770" patent. In accordance with the label on the "Breathable Sealing Membrane", it is intended to be used with said "Microwell Plate", also sold by Nalge Nunc, International.

Even if the "Breathable Sealing Membrane" is sold alone, such sale constitutes inducing of infringment, in accordance with 35 USC §271(b), and contributory infringement, in accordance with 35 USC §271(c), since it is intended for use with a multiwell plate, whether or not the membrane and plate are sold together.

Please note also that the manufacture of the "Breathable Sealing Membrane" in the United States is an infringement of the "770" patent, whether or not the manufactured product is sold in the United States. Conversely, the sale of the "Breathable Sealing Membrane" in the United States constitutes and infringement, regardless of where the product has been manufactured.

You were constructively notified of this infringement upon issue of the "770" patent, whether or not you were actually aware of said issue. However, you were formally notified of your infringement by a letter, dated November 6, 1999, written by Mr. Mark Fins, president of Diversified Biotech, Inc., and addressed to Mr. Steven Tomassi. See Exhibit B. As a result, there is no possible question that your infringement has been willful.

You are hereby ordered to immediately cease and desist the manufacture, sale, and use, in the United States, of any and all products which infringe on the "770" patent, either directly or indirectly. In addition, you are ordered to notify any third parties involved in any way with your use and promotion of your infringing products that the manufacture, sale, and use of these products will and must cease immediately. These third parties include, but are not limited to, Minnesota Mining and Manufacturing Company (3M). Your are further ordered to destroy any and all products presently in your inventory which infringe upon the "770" patent, and to notify all of your distributors and resellers to do likewise.

You are further ordered to notify this office that you are, and will remain in compliance with these orders.

If the above steps are not taken forthwith, my client has authorized me to take whatever steps deemed necessary to protect his interests. These may include filing of a complaint in Federal District Court, as well as procuring an injunction against your manufacture, use, and sale of the patented invention licensed by my client. If you are found liable of patent infringement, you may be assessed treble damages in accordance with 35 U.S.C. §284, and attorney's fees, in accordance with 35 U.S.C. §285.

Please contact me at the above address or telephone number at your earliest convenience to resolve this matter.

Yours truly,

Mark P. White
MPW/ag encl. cc: Mark Fins, Diversified Biotech, Inc.