1. INTENDED USE

The CycleTEST™ PLUS DNA Reagent Kit provides a set of reagents for isolating and staining cell nuclei from surplus fresh or frozen solid tissue specimens or cell suspensions. Flow cytometric analysis of differentially stained normal and tumor cells is used for research in the identification of abnormal DNA stemlines and to estimate the DNA index (DI) and cell-cycle phase distributions of these stemlines.

2. SUMMARY AND EXPLANATION

Uniform suspensions of single nuclei are prepared for DNA staining and flow cytometric analysis from surplus solid tissue specimens or cell suspensions. The in vitro fine-needle aspiration (FNA) technique ensures broad sampling of the tumor to maximize the extraction of malignant cells that may be present in the specimen, while minimizing cellular debris.

Flow cytometric DNA stemline analysis is being used for characterizing clonal diversity and researching the potential for clinical progression of these human neoplasms. 1-12

3. PRINCIPLES OF THE PROCEDURE

The method involves dissolving the cell membrane lipids with a nonionic detergent, eliminating the cell cytoskeleton and nuclear proteins with trypsin, digesting the cellular RNA with an enzyme, and stabilizing the nuclear chromatin with spermine. 13,14 Propidium iodide (PI) is stoichiometrically bound to the clean, isolated nuclei which are then run on a flow cytometer equipped with electronic doublet-discrimination capability. 15,16 Propidium iodide-stained nuclei emit fluorescent light primarily at wavelengths between 580 and 650 nm. The FACScan’s fluorescence 2 (FL2) detector, equipped with
a 585/42 bandpass filter, is used to analyze light emitted between 564 and 606 nm by the stained cells.

The resulting fluorescence histograms may be analyzed to detect the presence of an abnormal DNA stemline (DNA aneuploidy). Normal cells obtained from the same tissue or peripheral blood mononuclear cells (PBMCs) can be mixed with the sample in a second tube before staining and used as a reference to determine the degree of DNA content aberration. The DI is obtained by dividing the mode (or mean) of the relative DNA content of the abnormal G<sub>0</sub>/G<sub>1</sub> population by the mode (or mean) of the normal G<sub>0</sub>/G<sub>1</sub> population. The coefficient of variation for each G<sub>0</sub>/G<sub>1</sub> peak is also reported.\textsuperscript{17}

4. REAGENTS __________________________________________

Reagents Provided, Sufficient for 40 Tests

The CycleTEST PLUS DNA Reagent Kit contains four components:

Solution A (10 mL)
Contains trypsin in a spermine tetrahydrochloride detergent buffer for the enzymatic disaggregation of the solid tissue fragments and digestion of cell membranes and cytoskeletons.

Solution B (8 mL)
Contains trypsin inhibitor and ribonuclease A in citrate-stabilizing buffer with spermine tetrahydrochloride to inhibit the trypsin activity and to digest the RNA.

Solution C (8 mL)
Contains propidium iodide (PI) and spermine tetrahydrochloride in citrate stabilizing buffer. The PI stoichiometrically binds to the DNA at a final concentration of at least 125 µg/mL.
Buffer Solution (3 vials, 50 mL per vial)
Contains sodium citrate, sucrose, and dimethyl sulfoxide (DMSO) for the collection and/or freezing of cell suspensions.

Precautions

1. For research use only. Not for use in diagnostic or therapeutic procedures.

2. When stored at -18°C, the reagents are stable until the expiration date shown on the CycleTEST PLUS kit label. Do not use after the expiration date. The thawed reagents are stable for 1 month when stored at 2° to 8°C.

3. The reagents should not be refrozen after thawing. Do not expose reagents to direct light during storage or during incubation with cells.

4. Reagents should not be heated to 37°C although they may be briefly exposed to a 37°C water bath in the process of thawing.

5. Solution C (PI) should be protected from light and kept ice cold (2° to 8°C).

6. Incubation times, centrifugation times, or temperatures other than those specified may be a source of error.

7. For optimal results, analyze stained specimens within 3 hours of staining.

8. Alteration in the appearance of the reagents, such as precipitation or discoloration, indicates instability or deterioration. In such cases the reagent(s) should not be used.
9. **WARNING:** Solutions A, B, and C contain spermine tetrahydrochloride, which is irritating to the skin and mucous membranes. Solution C contains propidium iodide, which is a suspected mutagen. The citrate Buffer Solution contains DMSO, a possible teratogen. For all solutions, avoid contact with eyes, skin, and clothing. If contact occurs, flush affected areas immediately with water. Consult a physician if contact with eyes occurs. Avoid breathing vapors and wash surfaces thoroughly after handling.

10. **WARNING:** All specimens and any materials in contact with the specimen should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. Since no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, the specimen should be handled and disposed of at the Biosafety Level (BSL) 2 as recommended for any potentially infectious human serum or blood specimen. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.

5. **INSTRUMENT**

The CycleTEST PLUS DNA Reagent Kit is designed to prepare samples for DNA analysis on a flow cytometer equipped with appropriate computer hardware, software, and gating electronics. The flow cytometer must have linear fluorescence amplification capabilities with forward-scatter (FSC) and side-scatter (SSC) detection.

The flow cytometer should be equipped with a light source providing excitation in the blue-to-green range. For PI excitation, an argon-ion laser emitting at 488 nm is optimal. The following instrument system is recommended:
• Becton Dickinson FACScan™ or FACSort™ flow cytometer system equipped with a doublet discrimination module (DDM) or FACS Vantage™ flow cytometer equipped with pulse processing, and three-color fluorescence detection and two-parameter light-scatter detection (Becton Dickinson Catalog No. 34001010).

• On the Macintosh platform, Becton Dickinson CELLQuest™ software, version 1.0 (or later), with the DNA Experiment Document and appropriate analysis software. For detailed information on use, refer to the CELLQuest Software User’s Guide.

• On the Hewlett-Packard (HP) platform, Becton Dickinson CellFIT™ software, version 2.0 (or later) or 2.01.2 (or later). For detailed information on use, refer to the CellFIT Software User’s Guide.

6. SPECIMEN COLLECTION AND PREPARATION

Solid Tissue

NOTE: Process surplus tissue immediately upon receipt.

If the solid tissue specimen has been frozen, allow tissue to thaw at room temperature (20° to 25°C) before performing aspiration.

Prepare specimen collection tubes by labeling them with the appropriate identification number and adding 1 mL of citrate Buffer Solution into each tube.

1. For specimens smaller than 0.5 cm: Place smaller piece or pieces of tumor inside dialysis tubing and fold over once, parallel with one of the lateral creases in the tubing. Then fold each corner and fix to a styrofoam block with 27-gauge needles or stainless steel pins. The tumor is now immobilized and enclosed. Perform aspiration. (See Fine-Needle Aspiration Procedure in Section 7, Procedure.)

2. For specimens larger than 0.5 cm: The samples can be processed without wrapping the specimen in
tubing. Affix tumor tissue directly to a styrofoam block to anchor the sample for aspiration. Perform aspiration. (See Fine-Needle Aspiration Procedure in Section 7, Procedure.)

**Cell Suspensions**

Samples received containing cells already in suspension from tissue culture or body fluids may also be used for DNA analysis.

1. Place cell suspension into a 17 x 100-mm tube.

2. Centrifuge for 5 minutes at 300 x g at room temperature (20° to 25°C).

3. Aspirate the supernatant leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet. Add 1 mL of Buffer Solution and resuspend the cells by gently vortexing at low speed.

4. Centrifuge for 5 minutes at 300 x g at room temperature (20° to 25°C).

5. Aspirate the supernatant leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet. Resuspend the pellet in 1 mL of Buffer Solution by gently vortexing at low speed.

6. Centrifuge for 5 minutes at 300 x g at room temperature (20° to 25°C).

7. Aspirate the supernatant leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet. Resuspend the pellet in 1 mL of Buffer Solution by gently vortexing at low speed.

8. Count the cells by standard laboratory methods using a hemacytometer. Adjust the concentration to 1.0 x 10^6 cells/mL with Buffer Solution.
9. Cells are now ready for immediate staining and flow cytometric analysis. Cells may also be frozen for later testing. Refer to Freezing Procedure in Section 7, Procedure.

7. PROCEDURE ______________________________

Reagents Provided

See Reagents Provided and Precautions in Section 4, Reagents.

Reagents and Materials Required but Not Provided

For Specimen Preparation:
1. 17 x 100-mm capped polypropylene tubes (Becton Dickinson Falcon™ Catalog No. 2059).

2. 2 x 10 x 10-cm styrofoam block.

3. 27-gauge x 1.25-inch needles (Becton Dickinson Catalog No. 5136 in the US or Catalog No. 002200 in Europe) or stainless steel pins to anchor tissue to the block.

4. Dialysis tubing (Baxter Catalog No. D1614-5) to immobilize small samples.

5. Freezer-safe polypropylene tubes (1 mL) with air-tight screw caps (Cole-Parmer Catalog No. YB-06754-95 or Nunc Catalog No. 366656).

6. Transfer pipets or disposable pipets.

7. 20-cc syringes (Becton Dickinson Catalog No. 9694).

8. 25-gauge x 1.5-inch hypodermic needles (Becton Dickinson Catalog No. 5127) for tissue aspiration.

10. Low-speed centrifuge (300 x g) with swinging-bucket rotor and tube carriers for the 17 x 100-mm and 12 x 75-mm sample tubes.

11. Vacuum aspirator with trap.

12. Vortex mixer.

**For Cell Freezing:**
1. Dry ice.
2. 99% ethanol.

**For Cell Staining:**
1. 12 x 75-mm capped polypropylene tubes (Becton Dickinson Falcon Catalog No. 2063).

2. 2-mL disposable pipets (Becton Dickinson Falcon Catalog No. 7507) or Finnpipette digital pipet (LabSystems Catalog No. 4027040).

3. Ficoll-Paque® separation medium (Pharmacia Catalog No. 11-A-137-07).

4. Hemacytometer and microscope.

5. 50-µm Nitex® nylon mesh (TETKO® Catalog No. 3-50/21) or 12 x 75 mm tube with 35 µm cell strainer cap (Becton Dickinson Falcon Catalog No. 2235).

6. Ice bath.

7. Aluminum foil.

8. Disposable Kimwipe tissues (Kimberly-Clark Catalog No. 34155).

9. 200-µL to 1000-µL adjustable micropipettor and disposable tips (Pipetman™ or equivalent).

10. Sheath fluid (Haema-Line®2 diluent in US, Serono-Baker Catalog No. 49-002-497-000 or FACSFlow™ in Europe and Canada, Becton Dickinson Catalog No. 342003 [95-2003]).
NOTE: Using phosphate-buffered saline (PBS) instead of sheath fluid in the FACScan may result in different performance characteristics.

**For Instrument Setup:**
DNA QC Particles (Becton Dickinson Catalog No. 349523 [95-0023])

Refer to the DNA QC Particles package insert for instructions for use.

**Fine-Needle Aspiration Procedure**

NOTE: Areas of the tumor specimen appropriate for sampling should be indicated by a trained pathologist. Use a new syringe and needle for each tumor specimen to avoid cross-contamination of samples. If the specimen is not to be processed immediately, collect cells in freezer-safe vials and store frozen as described below in the Freezing Procedure.

1. Assemble syringe into pistol-grip syringe holder and attach a 25-gauge x 1.5-inch needle.

2. Introduce the needle tip into the tumor and when positioned, pull back gently on the syringe plunger to produce a vacuum. In larger samples, pass the needle through as many aspects of the mass as possible, without breaking the vacuum. In some laboratories, quadrant areas are designated for multiple aspirations in the tumor specimen to assure heterogeneous sampling. When processing small samples, attempt to aspirate as much tissue as possible.

3. When a visible amount of material appears in the needle hub (not in the barrel of the syringe), release the vacuum and then withdraw the needle.

4. Carefully expel the aspirated cells into 1 mL of Buffer Solution. Carefully rinse out cells remaining in the hub by aspirating Buffer Solution to fill the
needle hub only, not the syringe. Expel the wash buffer into the sample tube.

5. Enough cell suspension (approximately 2 drops) will remain in the needle to make a cytology slide. A drop or two of the recovered sample suspension may also be used. If sample size allows, a histopathology slide should also be prepared according to standard laboratory techniques. The suspension should be evaluated by a trained pathologist for adequate tumor representation using standard cytological methods.

6. Count cells using standard laboratory techniques. The dissolution of cell clumps during the subsequent enzyme and detergent steps may improve the recovery of nuclei compared to the original cell count. If clumps are present, estimate the cell number recovered from tissue fragments at this stage.

7. Repeat steps 2 through 4 until the cell concentration in the citrate buffer is at least $1.0 \times 10^6$ cells/mL. This should provide sufficient cells for both a test sample tube and a control tube.

**Controls**

**Peripheral Blood Mononuclear Cells (PBMCs)**

Control cells with known DNA content (PBMCs) may provide a reference point for determining the DI for a test sample. In order to use such a control, these cells must be prepared using the same reagents as the test sample and should ideally be added to an aliquot of the test sample before staining. The DI may be calculated from the ratio of the mode (or mean) of the $G_0/G_1$ peaks of the test and reference (PBMC) populations for a particular specimen.\(^{17}\)

To prepare PBMCs, purify the mononuclear cells using density-gradient centrifugation following the manufacturer’s instructions for use of the separation medium and then follow the procedure under Cell
Suspensions in Section 6, Specimen Collection and Preparation.

1. Purify mononuclear cells using density-gradient centrifugation; follow the manufacturer’s instructions for use of the separation medium.

2. Place the white blood cell suspension into a clean 17 x 100-mm tube.

3. Add 5 mL of Buffer Solution and resuspend the cells by gently vortexing at low speed.

4. Centrifuge for 5 minutes at 300 x g at room temperature (20° to 25°C).

5. Aspirate the supernatant leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet. Resuspend the pellet in 1.5 mL of Buffer Solution by gently vortexing at low speed.

6. Centrifuge for 5 minutes at 300 x g at room temperature (20° to 25°C).

7. Aspirate the supernatant leaving approximately 50 µL of residual fluid in the tube to avoid disturbing the pellet. Resuspend the pellet in 1-mL Buffer Solution by gently vortexing at low speed.

8. Count the cells using standard laboratory methods with a hemacytometer. Adjust the concentration to 1.0 x 10^6 cells/mL with Buffer Solution.

9. Cells are now ready for immediate staining and flow cytometric analysis. Cells may also be frozen for later testing. Refer to Freezing Procedure below.

**Freezing Procedure**

If the sample is not to be processed immediately, transfer the cell suspension in Buffer Solution to a freezer-safe polypropylene tube with a screw cap and freeze rapidly in a mixture of dry ice and 99% ethanol (−80°C). Store frozen samples at −80°C. Before analysis, thaw the
samples rapidly in a waterbath at 37°C, without allowing the sample material to reach 37°C.

**Staining Procedure**

*CAUTION:* Powder-free latex gloves should be worn to protect skin from contact with reagents.

NOTE: Use Solution A and B at room temperature (20° to 25°C). Solution C should be kept cold (2° to 8°C) and protected from light.

1. The staining procedure for DNA ploidy analysis requires a test sample of 5.0 x 10⁵ cells. An additional sample tube of the specimen mixed or “spiked” with PBMCs should be prepared and used as a control. Use at least a 2:1 ratio of tumor cells to PBMCs.

2. Centrifuge the cell suspensions at 400 x g for 5 minutes at room temperature (20° to 25°C). Carefully decant all the supernatant, and tap off the last drop onto a tissue.

3. Add 250 µL of Solution A (trypsin buffer) to each tube and gently mix by tapping the tube by hand. *Do not vortex.*

4. Allow Solution A to react for 10 minutes at room temperature (20° to 25°C). Do not remove Solution A.

5. Add 200 µL of Solution B (trypsin inhibitor and RNase buffer) to each tube and gently mix by tapping the tube by hand. *Do not vortex.*

6. Incubate with Solution B for 10 minutes at room temperature (20° to 25°C). Do not remove Solution A and B.

7. Add 200 µL of cold (2° to 8°C) Solution C (propidium iodide stain solution) to each tube. Gently mix as above and incubate for 10 minutes in the dark on ice or in the refrigerator (2° to 8°C).
8. Filter the sample through 50-µm nylon mesh into a labeled 12 x 75-mm tube or use 35-µm cell strainer cap and filter into 12 x 75-mm tube.

9. The samples are now ready to be analyzed on the flow cytometer. Cap or cover the prepared tubes and store at 2° to 8°C in the dark until flow cytometric analysis.

10. Run samples on the flow cytometer within 3 hours after addition of Solution C. After storage, be sure to mix the sample in the tubes by tapping the tube by hand to resuspend the cells.

**Quality Control**

For optimal results on the HP platform, Becton Dickinson recommends using the DNA QC Particles and CellFIT software. For optimal results on the Macintosh platform, Becton Dickinson recommends using the DNA QC Particles and CELLQuest software with the DNA Experiment Document. Both methods use the DNA QC particles to set the photomultiplier tube (PMT) voltages and check instrument resolution and linearity on the FACScan or FACSort flow cytometer. For instrument setup, please refer to the Becton Dickinson DNA QC Particles package insert.

Becton Dickinson recommends that a control tube be run with each test sample. See Controls in Section 7, Procedure.

It is recommended that the samples be run at an acquisition rate of at least 60 events per second. For samples prepared for DNA analysis according to this method, the FACScan flow rate should be set on LO.

Leave the flow cytometer on RUN between samples to allow the sample injection port to backflush. Before placing the flow cytometer’s fluidics control knob into STANDBY mode, ensure that fluidics are rinsed thoroughly to prevent debris from adhering to the inside of the flow cell.
The histograms must be analyzed by an appropriate DNA analysis program.

8. RESULTS

Results (Figures 1–4) were obtained by using CycleTEST PLUS DNA reagents and CellFIT software on a DDM-equipped FACScan flow cytometer.

Becton Dickinson recommends that at least 20,000 list-mode data events be acquired for each sample. More data events may be required for samples containing several smaller subpopulations.

Figure 1. Peripheral blood mononuclear cells (PBMCs): FL2-Width versus FL2-Area dot plot showing a singlet gate, which excludes aggregates. PBMCs may be mixed with tumor samples as an internal control.
Figure 2. FL2-A DNA histogram of PBMCs, which has been gated to exclude aggregates.

Figure 3. Breast tumor tissue: FL2-Width versus FL2-Area dot plot showing a singlet gate, which excludes aggregates.
Figure 4. FL2-A DNA histogram of breast tumor tissue, prepared by in vitro FNA, which has been gated to exclude aggregates.
9. LIMITATIONS

1. The information obtained from this kit must be combined with other relevant information. Interpretation of this information by a medically qualified diagnostian is necessary.

2. The user must ensure the inclusion of tumor cells by carefully adhering to the sample preparation procedure described in this document. Light microscopy confirmation must be performed to ensure that the suspension adequately represents the tumor under study. The presence of malignant cells must be confirmed by a trained pathologist using a standard cytological evaluation of the tumor.

3. Performance characteristics have been determined with FACScan flow cytometers. Performance characteristics using other instruments have not been established.

4. Stained samples should be analyzed on the flow cytometer within 3 hours of staining.

5. Changing reagent volumes or incubation times from those specified may yield erroneous results. The reagents are formulated to appropriately process a specific number of cells. Using excessive numbers of cells may produce suboptimal results.

6. The fluorescence of PI is dependent on the pH and ionic strength of the solution. The use of PBS instead of sheath fluid in the FACScan may result in different performance characteristics.
## 10. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudden change or slow drift of diploid $G_0/G_1$ peak location.</td>
<td>1. Air bubbles or clogs in fluidics system.</td>
<td>1. Flush fluidics and clear lines of air.</td>
</tr>
<tr>
<td></td>
<td>2. Different tonicity of sheath and sample fluids.</td>
<td>2. Check quality of sheath fluid.</td>
</tr>
<tr>
<td></td>
<td>3. Insufficient PI staining.</td>
<td>3. Check concentration of nuclei and adjust.</td>
</tr>
<tr>
<td></td>
<td>4. Insufficient incubation of nuclei in PI.</td>
<td>4. Increase incubation time.</td>
</tr>
<tr>
<td>Presence of excess debris in fluorescence histogram.</td>
<td>1. Necrosis in original specimen.</td>
<td>1. Visually check the tissue specimen for necrosis.</td>
</tr>
<tr>
<td></td>
<td>2. Incomplete cell lysis.</td>
<td>2. Check suspension for incomplete lysis.</td>
</tr>
<tr>
<td></td>
<td>3. Particles in sheath fluid.</td>
<td>3. Clean system and replace sheath fluid and/or sheath filter if necessary.</td>
</tr>
<tr>
<td>Broadened peaks; Increased CVs.</td>
<td>1. Flow rate too high.</td>
<td>1. Fluidics control switch should be placed on LO.</td>
</tr>
<tr>
<td></td>
<td>2. Air bubbles in fluidics.</td>
<td>2. Check fluidics for air.</td>
</tr>
<tr>
<td></td>
<td>3. Necrotic cells included in sample.</td>
<td>3. Check for presence of necrotic cells and prepare another sample.</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Shifting of peaks due to loss of fluorescence.</em></td>
<td>1. Too many cells.</td>
<td>1. Check concentration of nuclei and adjust to optimum.</td>
</tr>
<tr>
<td></td>
<td>2. DNA not saturated with propidium iodide.</td>
<td>2. Stain another sample with correct concentration and volume of PI stain.</td>
</tr>
<tr>
<td></td>
<td>3. Residual bleach left in sample injection probe (SIP).</td>
<td>3. Ensure that fluidics are adequately rinsed after exposure to bleach.</td>
</tr>
<tr>
<td><em>Few or no events being acquired.</em></td>
<td>1. Incorrect mounting of sample tube.</td>
<td>1. Remount sample tube or check for cracks in sample tube.</td>
</tr>
<tr>
<td></td>
<td>2. No nuclei in sample.</td>
<td>2. Check for presence of nuclei in the sample using a microscope.</td>
</tr>
<tr>
<td></td>
<td>3. Sample line clogged.</td>
<td>3. Backflush and clean sample line.</td>
</tr>
<tr>
<td></td>
<td>4. Debris in flow cell.</td>
<td>4. Ensure that fluidics are rinsed thoroughly of sample fluid before placing the flow cytometer's fluidics control knob into STANDBY mode to prevent debris from adhering to the inside of the flow cell.</td>
</tr>
</tbody>
</table>
REFERENCES


**WARRANTY**

The product sold hereunder is warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. Becton Dickinson’s sole liability is limited to either replacement of the product or refund of the purchase price. Becton Dickinson is not liable for property damage, personal injury, or economic loss caused by the product.

**CUSTOMER SUPPORT INFORMATION**

Becton Dickinson Immunocytometry Systems
2350 Qume Drive
San Jose, California 95131-1807, USA
Ordering Information Tel (800) 223-8226
Customer Support Center Tel (800) 448-BDIS (2347)

Distributed by:
Becton Dickinson—
European HQ
Denderstraat 24
B-9320 Erembodegem-Aalst
Belgium
Tel (32) 53-720211
FAX (32) 53-720450

Becton Dickinson Canada, Inc
2464 South Sheridan Way
Mississauga, Ontario L5J 2M8
Canada
Tel (905) 822-4820
FAX (905) 855-1243

Nippon Becton Dickinson Company, Ltd
Shimato Building
5-34 Akasaka 8-chome
Minato-ku, Tokyo 107
Japan
Tel (81) 3-3403-9991
FAX (81) 3-3403-5008

Becton Dickinson Worldwide, Inc
30 Tuas Avenue, #2
Singapore 2263
Tel (65) 861-0633
FAX (65) 860-1590

7/94 23-2923-01
SUGGESTED SOURCES

Baxter Scientific Products Division, McGaw Park, IL (312) 689-8410.

Becton Dickinson, San Jose, CA (800) 223-8226; Europe: Erembodegem, Belgium (32) 53-720211.

Becton Dickinson, Rutherford, NJ (201) 460-4900; Erembodegem, Belgium (32) 53-720211.

Becton Dickinson, Lincoln Park, NJ (201) 628-1144; Erembodegem, Belgium (32) 53-720211.

Becton Dickinson, Franklin Lakes, NJ (201) 848-6500; Erembodegem, Belgium (32) 53-720211.

Cole Parmer, Chicago, IL (800) 323-4340.

L K LabSystems, Research Triangle Park, NC (800) 572-8270; Helsinki, Finland (358) 0-75821.

Nunc, Naperville, IL (313) 983-5700; Roskilde, Denmark (45) 42-359065.

Tetko, Inc., Monterey Park, CA (818) 289-9153; Swiss Silk Bolting Cloth Mfg. Zurich, Switzerland. (41) 12-889111.

Serono Baker Diagnostics, Allentown, PA (215) 264-2800 or Europe: Becton Dickinson, Erembodegem, Belgium (32) 53-720211.

NOTE: These are suggested sources. Other suppliers who provide equivalent products may be used.

FACS and Falcon are registered trademarks of Becton Dickinson and Company.
CycleTEST PLUS, FACScan, FACS Sort, FACS Vantage, CellFIT, CELLQuest, and FACSFlow are trademarks of Becton Dickinson and Company.
Macintosh is a registered trademark of Apple Computer, Inc.
Nitex and Tetko are registered trademarks of Tetko, Inc.
Finnpipette is a registered trademark of LK Labsystems.