Innovation on ‘Flow Cytometry’
Made easier and faster methods for Cell Analysis

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Scientists looking at protein expression see flow cytometry as a better way to study cells

- Scientists ask ‘What changes occur in protein expression when…’
  - Stem cells differentiate
  - Adult cells dedifferentiate (become cancer-like)
  - Cells divide, stop dividing, age, die
  - Cells are exposed to specific therapeutic candidates
  - Normal cells are compared to diseased cells

**Present:**
- Western Blotting
- Lysate of 10 million cells
- One protein per lysate
- Few samples/day
- Multiple manual steps
- Image based

**Growing Trend:**
- Flow Cytometry
- One cell at a time
- 4+ proteins per cell
- Hundreds of samples/day
- Automation compatible
- Quantitative
Flow Cytometry: The Basics

- Principles of flow cytometry and instrumentation
  - Fluidics, detectors, signal generation and processing, multiparametric data display
- Fluorescent probes and applications
- Multiple color design
What are you measuring?

- Light scatter intensity
- Fluorescence intensity
- Sample volume

The information that you infer from your data depends on your experimental design and your controls.
Light Scatter: The Interaction of Light with Cells

**Side scatter**
- Size related, but proportional to granularity, inclusions, surface structures, etc.

**Forward scatter**
- “Size” proportional to:
  - cross-sectional area of cells
  - refractive index
  - shape

Incident laser light
Side Scatter – How it works

- **Granulocytes**
  - Forward Scatter (FSC)
  - Side Scatter (SSC)

- **Monocytes**
  - Forward Scatter (FSC)
  - Side Scatter (SSC)

- **Lymphocytes**
  - Forward Scatter (FSC)
  - Side Scatter (SSC)
Fluorescence: light emitted by fluorescent dyes

- Photodiodes
  - less sensitive
  - good for light scatter

- Photomultiplier tubes (PMTs)
  - high sensitivity
  - good for fluorescence

FITC-labeled antibody binds surface antigen

488nm excitation

~520nm emission

Fluorophores
The key of traditional flow cytometer: sheath fluid
Weakness of traditional flow cytometry

- Complicated system: huge footprint
  - Big Sample volume
  - Large sheath fluid dependent
  - Waste production issue

- Higher requirement for usage of maintenance: troublesome
  - Daily maintenance
  - Difficult clog clearance, cell flow adjustment required
  - QC process
  - Special training and operator needed
  - High running cost (flow chamber, sheath fluid, waste disposal, washing buffer, reagent cost)

- Limited analysis function:
  - Software is not easily to be used
  - Limit of sample throughput
  - Accurate cell counting can not be guaranteed due to shortage of sheath fluid
  - Cell analysis limitation: can not characterize the apoptotic cells
## Guava Technologies micro-capillary cytometry

Patented Microcapillary Design Replaces Sheath Flow

### Guava-Patented Micro-capillary System

<table>
<thead>
<tr>
<th>Sheath Fluid: None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste: &lt; 80 mL per 8 hr run</td>
</tr>
<tr>
<td>Typical # Cells Per Protocol: 2,000 – 10,000 cells/Test</td>
</tr>
</tbody>
</table>

### Traditional Sheath Fluid System

<table>
<thead>
<tr>
<th>Sheath Fluid: ~10 mL/Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste: 8,000 mL per 8 hr run</td>
</tr>
<tr>
<td>Typical # Cells Per Protocol: 100,000 – 1,000,000 cells/Test</td>
</tr>
</tbody>
</table>

### Advantages vs. Other Flow Platforms

- Significantly smaller footprint
- Lower number of cells required
- Less waste and reagent consumption
- Lower cost
Guava Cytometry Platform

Significant small footprint

Less waste and reagent consumption - No sheath fluid or alignment and Lower number of cells required

Lower cost - Easy to maintain and operate and no need for dedicated operator

Minimal downtime
• Easy to maintain and operate
• No sheath fluid or alignment
• No need for dedicated operator
• Minimal downtime
• Small footprint
Fixed flow cell

• No flow cell alignment is required
  – Flow cell is user-replaceable
  – Flow cell is self-aligning

• No user-performed hardware adjustments required
  – Optics and fluidics systems are fixed

Guava-Patented Micro-capillary System
It is easier to clean Clog

<table>
<thead>
<tr>
<th>Traditional flow cytometer</th>
<th>Guava flow cytometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleaning flush—sheath fluid</td>
<td>Quick cleaning—water/ICF</td>
</tr>
<tr>
<td>Off-line Syringe flush</td>
<td>In-line/off-line Syringe flush</td>
</tr>
<tr>
<td>Alignment Check</td>
<td>Alignment Check Free</td>
</tr>
<tr>
<td>Sheath fluid pressure check</td>
<td>No Sheath fluid Needed</td>
</tr>
</tbody>
</table>
Guava is green

what a big difference
“build in” to realize high throughput
Compatible with automatic system

• Works with most physically compatible robotic arms and/or liquid handling stations

• Robotic arms
  – Zymark Twister II
  – Hudson PlateCrane
  – Hamilton MicroLab Swap
  – Others

• Liquid Handling Stations
  – BioMek Series
  – Perkin Elmer MultiProbe
  – Tecan Liquid Handlers with RoMa Arm
  – Others

GuavaLink Software provides a general interface for many automation systems
Capable of full walkaway automation
Daily QC is Easy and Automated

- Guava Check Beads
  - Daily QC of system performance
  - Levy-Jennings graphs automatically calculated

Guava Check Beads: Daily QC of system performance
Daily maintenance

• Clean and Shut Down at end of day
  – Automates cleaning of capillary fluidics in
    ~15 minutes using ICF
• Empty waste container at end of day (<80 mL)
  – Minimal waste disposal requirements
Software is so easier to handle
Smaller, stronger – Guava flow cytometry

• Simply system: tiny footprint
  – Small sample volume, Easy sample preparation
  – No need for sheath fluid: 1/10~1/100 of conventional sample, 2,000-10,000 cells/Test
  – Less waste production: 1/100 of conventional waste

• System maintenance:
  – Fixed flow cell and cell flow adjust automatically
  – Easy to clear Clog
  – Easy to use daily QC tool: Guava Check Beads
  – No special operator need and realize personal
  – Low cost (No sheath fluid, micro-capillary, less waste, easy clean and less reagent)

• Stronger analysis software
  – Use friendly software especially the software module
  – Compatible with different kinds of plate and EP
  – Absolute cell counting
  – Can characterize the percentage of Apoptotic cells
Small Sample Volumes Allow for Repeat Tests on the Same Animal: A single drop of blood

Guava’s unique microcapillary technology allows for small sample volumes of less than 5 μL to be used per test; a single drop of blood (~50 μL) is thus sufficient to perform up to ten tests. Such small sample volumes allow researchers to obtain longitudinal information (repeat tests on the same animal) over the course of the study in a way not previously available using traditional methods.

Dr. Keith Crawford, M.D., Ph.D., Director of Genomic and Proteomic Research in the Center of Molecular Orthopedics at Brigham and Women’s Hospital/Harvard Medical School and the Dept. of Defense, commented, “The introduction of the microcapillary technology has changed the way we can plan and carry out trials in animal models. Previously, we had to use a new mouse for each time point due to the large volumes of blood required per sample. Now, we can use the same mouse for multiple experiments, using just a drop of blood each time. This provides us with information that we were not able to get before, such as time dependent changes in immune function in the same mouse.”

Guava’s microcapillary technology is well suited for working with precious and limited samples,” said Todd Christian, Vice President of Commercial Operations. “This capability differentiates us within the life sciences market and provides more relevant data researchers could not obtain using traditional methods.”
Guava platform: Choose your right platform

<table>
<thead>
<tr>
<th></th>
<th>PCA</th>
<th>EasyCyte Mini</th>
<th>PCA-96</th>
<th>EasyCyte Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (with PC)</td>
<td>35 lbs (11.4kg)</td>
<td>50 lbs (22.7kg)</td>
<td>75 lbs (36kg)</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>9&quot; (23cm)</td>
<td>9&quot; (23cm)</td>
<td>14&quot; (35cm)</td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>14&quot; (36cm)</td>
<td>18&quot; (46cm)</td>
<td>23&quot; (56cm)</td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td>13&quot; (32cm)</td>
<td>15.5&quot; (39cm)</td>
<td>16&quot; (40cm)</td>
<td></td>
</tr>
</tbody>
</table>
• Blue (EasyCyte) or Green (PCA) laser excitation
• Up to 6 parameters: FSC, SSC, Green, Yellow, Red, Near Infrared
Guava platform specification

<table>
<thead>
<tr>
<th></th>
<th>PCA Single Sample</th>
<th>PCA-96 96 wells/10 tubes</th>
<th>EasyCyte Mini Single Sample</th>
<th>EasyCyte Plus 96 wells/10 tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser, Excitation</td>
<td>532 nm</td>
<td>532 nm</td>
<td>488 nm</td>
<td>488 nm</td>
</tr>
<tr>
<td>FCS Size Detection</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>580/20 nm (PCAs)</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>583/26 (ECs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow/Orange</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>675/25 nm (PCAs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>680/30 nm (ECs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>525/30 nm Filter Green</td>
<td>–</td>
<td>–</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>785/70 nm Filter Near Infrared</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>√</td>
</tr>
<tr>
<td>SSC Granularity</td>
<td>–</td>
<td>–</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>
## Guava assay - Dye based assay

<table>
<thead>
<tr>
<th></th>
<th>EasyCyte Plus</th>
<th>EasyCytemini</th>
<th>PCA-96</th>
<th>PCA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample Format</strong></td>
<td>Tube Sampling</td>
<td>0.5ml and 1.5ml</td>
<td>1.2ml and 1.5ml</td>
<td>0.5ml and 1.5ml</td>
</tr>
<tr>
<td></td>
<td>96-well Plates</td>
<td>✓</td>
<td>–</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Minimal Sample Size (volume&lt;20ul)</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Waste generation in 8 hours of continuous use</strong></td>
<td>&lt;40ml</td>
<td>&lt;40ml</td>
<td>&lt;40ml</td>
<td>&lt;30ml</td>
</tr>
<tr>
<td><strong>ViaCount</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Cell Cycle</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Cell Growth</strong></td>
<td>✓</td>
<td>✓</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Antibody Quantitation</strong></td>
<td>✓</td>
<td>✓</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Cell Tracking</strong></td>
<td>CellToxicity</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>CellPaint</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Caspases 3/7, 8</strong></td>
<td>✓</td>
<td>✓</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Apoptosis</strong></td>
<td>Nexin-early Apoptosis (Annexin-V binding)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>TUNEL-Late Apoptosis</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>MitoPotential</td>
<td>✓</td>
<td>✓</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Multicaspase-mid-</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
## Common fluorophores – know these!!

<table>
<thead>
<tr>
<th>Category</th>
<th>PM1 (Orange) Add nm</th>
<th>PM2 (Red)</th>
<th>PM3 (Green)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorophores Conjugated to an Ab</td>
<td>PE TRITC</td>
<td>PE-Cy5</td>
<td>FITC</td>
</tr>
<tr>
<td>Dead Cell Dyes (DNA staining)</td>
<td>PI</td>
<td>7-AAD</td>
<td></td>
</tr>
<tr>
<td>Live Cell Dye (DNA Staining)</td>
<td></td>
<td>LDS751</td>
<td></td>
</tr>
<tr>
<td>Fluorescent Proteins</td>
<td></td>
<td></td>
<td>GFP</td>
</tr>
<tr>
<td>Cell Tracking Dyes</td>
<td>CFSE</td>
<td></td>
<td>CFSE (optimum)</td>
</tr>
</tbody>
</table>
Smaller, stronger

Assay Outputs Delivered By The Guava Platform
Software is so easier to handle
1. **Cell counting and viability**: Guava ViaCount® Assay

2. **Guava kits-Dye based**
   - **Apoptosis**
   - Early: Guava Nexin® Assay, Guava Mitochondrial Depolarization Assay
   - Mid-stage: MultiCaspase, Caspase 3/7, Caspase 8
   - Late-stage: Guava TUNEL Assay
   - **Cell cycle**: Guava Cell Cycle Assay
   - **Cell tracking**: Guava Cell Toxicity Assay & Guava Cell Growth Assay

3. **Open platform- Home-brew antibodies and Flow cellect™ kits**
Software is so easier to use
Guava ViaCount® Assay

Assay Principle
- Based on membrane permeability of 2 DNA-binding dyes
- Counts by volumetric means; not beads or impedance

Preparation
- Mix and Read: 5-15 Minute Incubation
- Guava PCA: Prepared all in One tube
- Guava PCA-96 & EasyCyte: Prepared All in One Plate or Tube option

Assay is Accurate, Fast, Easy
No Wash. Mix and Read Format.
How Guava ViaCount Works: LDS751

First Fluorochrome Enters All Cells, Binds to DNA, and Fluoresces at Its Characteristic Wavelength

ViaCount Reagents Work with Most Cell Types
How Guava ViaCount Works: PI

Second Fluorochrome Enters Only Dead Cells, Binds to the DNA, Fluoresces at a Different Wavelength

ViaCount Reagents Work with Most Cell Types
ViaCount Typical result

One System for Multiple Outputs Related to BioAnalysis and Cell Health

PM1: Viability Indicator

- Viable cell (red FL only)
- Apoptotic cell (red FL + dim yellow)
- Dead cell (red FL + yellow FL)
- Debris (unlabeled)

PM2: Nuclear Indicator

- Viable cell
- Apoptotic cell
- Dead cell
- Debris (unlabeled)
Guava ViaCount Assay- to identify the percentage of apoptotic cells

Uses a more robust method for assessing cell counts and viability (two specific DNA binding dye) Provides apoptotic fraction, in addition to cell counts and viability assessment.
Absolute viacount analysis

- Volume = 200 nl
- Flow Rate = 1 µl / second
- Probe Volume = 200 pl
- Flow Rate = 1 µl / second
- Volume = 50-100 mm
- 0.1 mm
- 20 µm
Software is so easier to handle
Guava Apoptosis Assay Suite

Programmed Cell Death

- Fluorescent readout
- Single cell-based analysis of intact cells
- Microplate- and tube-based assays
- 2nd reagent added to monitor later stages
- Absolute cell counts
How the Guava Nexin Assay Works

Measures early apoptotic cells & late apoptotic or dead cells

- PS residues exposed on extracellular membrane during apoptosis are bound by Annexin V
- 7-AAD enters cells when membranes are compromised in either dead or late apoptotic cells
Guava Nexin® Assay

Assay Principle

- Determines number of cells undergoing apoptosis by the binding of Annexin V-PE to phosphatidylserine, a membrane component translocated to the cell surface during apoptosis
- Determines number of late apoptotic and dead cells based on loss of membrane integrity

Preparation

- **Mix and Read Assay**: Add reagent and incubate 20 min
- **Total Assay Prep Time**: <30 minutes
- **Guava Nexin Assay**: Works on ANY Guava System
- **Acquisition Time on Guava Systems**:
  - PCA & Mini: <1 minute / sample
  - PCA-96/EasyCyte Plus: ~45 minutes / microplate
Guava Nexin Assay at a Glance

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Based on Annexin V binding</td>
<td>Annexin V is a standard marker for early apoptosis</td>
</tr>
<tr>
<td>Uses 7-AAD counter stain</td>
<td>Reliably discriminates dead cells from apoptotic cells</td>
</tr>
<tr>
<td>2x10⁴ cells in 100 µL volume</td>
<td>Minimal # of cells required</td>
</tr>
<tr>
<td>Dedicated Guava Nexin Software Module</td>
<td>Works on all Guava Systems</td>
</tr>
<tr>
<td>• 4 quadrant dot plot analysis with user</td>
<td>Allows for discrimination of four populations: healthy, apoptotic and dead</td>
</tr>
<tr>
<td>controlled quadrant marker</td>
<td>cells and nuclei</td>
</tr>
<tr>
<td>• Automatic calculation for counts, % and</td>
<td>Instantaneous results: No need to configure software for assay</td>
</tr>
<tr>
<td>MFI</td>
<td></td>
</tr>
<tr>
<td>Pre-made reagent cocktail</td>
<td>Unlike all commercially available Annexin V kits, no need for reagent</td>
</tr>
<tr>
<td></td>
<td>preparation with Guava redesign kit</td>
</tr>
<tr>
<td></td>
<td>Time saving &amp; eliminates possible dilution errors</td>
</tr>
<tr>
<td>Protocols for non-adherent and adherent cells</td>
<td>Facilitates ease of use with established protocols</td>
</tr>
<tr>
<td>A true mix and read assay</td>
<td>Easy: Cells are directly stained in tube or microplate</td>
</tr>
<tr>
<td></td>
<td>Simplified: Staining and data acquisition can be completed in 1 hour</td>
</tr>
<tr>
<td></td>
<td>Convenient for routine screening use</td>
</tr>
</tbody>
</table>
• Acquisition view
  – Healthy cell culture results
• Analysis view
  – Induced cell culture results with gate enabled
Typical Nexin Results

Dedicated Guava Nexin Software Module: 4 quadrant dot plot analysis with user controlled quadrant marker. Automatic calculation for counts, % and MFI.
Software is so easier to handle
FlowCellect Assays Provide Essential Stem Cell Information

Mouse ESC Characterization

Human ESC Characterization

Rodent NSC Characterization

Human ESC Surface Characterization

Assay Outputs Delivered By The Guava Platform

Plot2: Gated by: Gate 1
* Any other cell types that express an incomplete set of the defined markers may not qualify as stem cells.
### Stem cell FlowCellect™ kit format

<table>
<thead>
<tr>
<th>Number of assays per kit:</th>
<th>25 tests for 3 color stem cell kits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell count range per assay:</td>
<td>150,000~250,000 cells per assay</td>
</tr>
<tr>
<td>Max. cell concentration:</td>
<td>500 cells/µl</td>
</tr>
<tr>
<td>Assay volume:</td>
<td>100 µl (plate) - 500 µl (single tube)</td>
</tr>
</tbody>
</table>

**Kit Components (reagents):**

- Ab 1-FITC/Alexa488 conjugated (5 µl of 20x in a assay, enough for 25 assays)
- Ab 2-PE conjugated (5 µl of 20x in a assay, enough for 25 assays)
- Ab 3-PE/CY5 conjugated (5 µl of 20x in a assay, enough for 25 assays)
- Isotype control for Ab 1-FITC/Alexa488 conjugated (5 µl of 20x in a assay, enough for 5 assays)
- Isotype control for Ab 2-PE conjugated (5 µl of 20x in a assay, enough for 5 assays)
- Isotype control for Ab 3-PE/CY5 conjugated (5 µl of 20x in a assay, enough for 5 assays)
- 10X Wash buffer = 10X PBS-(13 mL)
- 4X Fixation solution = 8% paraformaldehyde-(10 mL)
- 1X Permeabilization Buffer* = 1X PBS+0.1% Triton-X 100-(10 mL)
- 5X Assay buffer = 5X PBS+0.25% sodium azide+10% FCS-(50 mL)
- Cell strainer (60 µm) cat# SCNY00060-(25)

* Component for intracellular kits only

**Kit Packaging and Literature:**

- Product datasheet/Instruction manual
- MSDS
- Kit Box
- Insert, COA
FlowCellect Stem Cell Kits Advantages

- Features:
  1. 3 parameter antibody labeling
  2. Fluorophore conjugated primary antibodies
  3. Prediluted and optimized reagents
  4. Complete set of reagents for the protocol
  5. Cell strainer included

- Benefits:
  1. Ensures specific labeling of stem cells
  2. Minimizes cell loss; Limits need for extra wash steps
  3. Minimal assay development and optimization required
  4. All buffers are included for the required staining steps
  5. Reduces cell clumping - duplets

Fully Validated and Optimized Kit for Flow Cytometry Analysis
New FlowCellect Stem Cell Kits

- **Products available for Embryonic stem cell work:**
  1. **FCMEC25110:**
     - FlowCellect™ Mouse ESC Nuclear Marker Characterization Kit
  2. **FCHEC25102:**
     - FlowCellect™ Human ESC Nuclear Marker Characterization Kit
  3. **FCHEC25106:**
     - FlowCellect™ Human ESC (TRA-1-60) Surface Marker Characterization Kit
  4. **FCHEC25104:**
     - FlowCellect™ Human ESC (HESCA-1) Surface Marker Characterization Kit
New FlowCellect Stem Cell Kits - Neural

- **Products available for Neural stem cell work:**
  - 5. FCRNC25112: FlowCellect™ Rodent NSC Characterization Kit (Neural)
  - 6. FCRNC25114: FlowCellect™ Rodent NSC Characterization Kit (Astrocyte)
Chemokines are the cytokines that may activate or chemoattract leukocytes. Each chemokine contains 65 ~ 120 amino acids, with molecular weight of 8 ~ 10 kD. Their receptors belong to G-protein-coupled receptors. Since the entry of HIV into host cells requires chemokine receptors, their antagonists are being developed to treat AIDS.
### FlowCellect™ Chemokine Receptor Kit Format

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of assays per kit:</td>
<td>100 tests</td>
</tr>
<tr>
<td>Cell count range per assay:</td>
<td>150,000~250,000 cells per assay</td>
</tr>
<tr>
<td>Max. cell concentration:</td>
<td>500 cells/µl</td>
</tr>
<tr>
<td>Assay volume:</td>
<td>100µl (plate)-500µl (single tube)</td>
</tr>
<tr>
<td>Kit Components (reagents):</td>
<td>Ab 1-Primary (20µl of 500x, enough for 100 assays)</td>
</tr>
<tr>
<td></td>
<td>Ab 2-PE conjugated secondary (20µl of 500x in a assay, enough for 100 assays)</td>
</tr>
<tr>
<td></td>
<td>Positive control cells (1 vial with 4 X 10^6 cells)</td>
</tr>
<tr>
<td></td>
<td>Negative control cells (1 vial with 4 X 10^6 cells)</td>
</tr>
<tr>
<td></td>
<td>Antibody Diluent</td>
</tr>
<tr>
<td></td>
<td>10X Wash buffer=10X PBS-(13mL)</td>
</tr>
<tr>
<td></td>
<td>4X Fixation solution=8% paraformaldehyde-(10mL)</td>
</tr>
<tr>
<td></td>
<td>5X Assay buffer=5X PBS+0.25%sodium azide+10%FCS-(50mL)</td>
</tr>
<tr>
<td>Kit Packaging and Literature:</td>
<td>Product datasheet/Instruction manual</td>
</tr>
<tr>
<td></td>
<td>MSDS</td>
</tr>
<tr>
<td></td>
<td>Kit Box</td>
</tr>
<tr>
<td></td>
<td>Insert , COA</td>
</tr>
</tbody>
</table>
FlowCellect Chemokine Receptor Kits Advantages

• Features:
  1. Flow cytometry validated primary antibodies
  2. Fluorescent conjugated antibody-based detection
  3. Control cells included
  4. Prediluted and optimized reagents
  5. Complete set of reagents for the protocol

• Benefits:
  1. Ensures specific labeling of Chemokine Receptors
  2. Less hazardous than radio-ligand binding assays.
  3. Defines protein concentration parameters
  4. Minimal assay development and optimization required
  5. All buffers are included for the required staining steps

Fully Validated and Optimized Kit for Flow Cytometry Analysis
11 FlowCellect™ chemokine receptor surface ID and quantification kits to be launched in 08

<table>
<thead>
<tr>
<th>Kit Description</th>
<th>Primary (1°) Antibody</th>
<th>Controls: fixed cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CCR1 Surface Expression Identification and Quantification</td>
<td>Monoclonal anti-human CCR1 (clone ID 53504)</td>
<td>ChemiScreen CCR1 (HTS005C) ChemiScreen WT (HTSCHEM1)</td>
</tr>
<tr>
<td>2 CCR2b Surface Expression Identification and Quantification</td>
<td>Monoclonal anti-human CCR2 (clone ID 48607)</td>
<td>ChemiScreen CCR2b (HTS007C) ChemiScreen WT (HTSCHEM1)</td>
</tr>
<tr>
<td>3 CCR3 Surface Expression Identification and Quantification</td>
<td>Monoclonal anti-human CCR3 (clone ID [5E8])</td>
<td>ChemiScreen CCR3 (HTS008C) ChemiScreen WT (HTSCHEM1)</td>
</tr>
<tr>
<td>4 CCR4 Surface Expression Identification and Quantification</td>
<td>Monoclonal anti-human CCR4 (clone ID 205410)</td>
<td>ChemiScreen CCR4 (HTS009C) ChemiScreen WT (HTSCHEM1)</td>
</tr>
<tr>
<td>5 CCR6 Surface Expression Identification and Quantification</td>
<td>Monoclonal anti-human CCR6 (clone ID 53103)</td>
<td>ChemiScreen CCR6 (HTS011C) ChemiScreen WT (HTSCHEM1)</td>
</tr>
<tr>
<td>6 CCR7 Surface Expression Identification and Quantification</td>
<td>Monoclonal anti-human CCR7 (clone ID 150503)</td>
<td>ChemiScreen CCR7 (HTS012C) ChemiScreen WT (HTSCHEM1)</td>
</tr>
<tr>
<td>7 CXCR1 Surface Expression Identification and Quantification</td>
<td>Monoclonal anti-human CXCR1 (clone ID 42705)</td>
<td>ChemiScreen CXCR1 (HTS001C) ChemiScreen WT (HTSCHEM1)</td>
</tr>
<tr>
<td>8 CXCR2 Surface Expression Identification and Quantification</td>
<td>Monoclonal anti-human CXCR2 (clone ID 5E8)</td>
<td>ChemiScreen CXCR2 (HTS002C) ChemiScreen WT (HTSCHEM1)</td>
</tr>
<tr>
<td>9 CXCR3 Surface Expression Identification and Quantification</td>
<td>Monoclonal anti-human CXCR3 (clone ID 49801)</td>
<td>ChemiScreen CXCR3 (HTS003C) ChemiScreen WT (HTSCHEM1)</td>
</tr>
<tr>
<td>10 CXCR4 Surface Expression Identification and Quantification</td>
<td>Monoclonal anti-human CXCR4 (clone ID 12G5)</td>
<td>ChemiScreen CXCR4 (HTS004C) ChemiScreen WT (HTSCHEM1)</td>
</tr>
<tr>
<td>11 CXCR6 Surface Expression Identification and Quantification</td>
<td>Monoclonal anti-human CXCR6 (clone ID 56811)</td>
<td>ChemiScreen CXCR6 (HTS054C) ChemiScreen WT (HTSCHEM1)</td>
</tr>
</tbody>
</table>
Millipore is developing co-labeled kits validated and optimized on the EasyCyte platform

- **Flow Cellect™ Kit Contents**
  - Pre-diluted (‘ready-to-use’) fluorescently-labeled primary antibodies (3 – 5 per kit)
  - Optimized buffers and other reagents
  - Sample preparation devices
  - Complete protocol

- **Value Proposition**
  - The scientist will only need to prepare the sample and follow the protocol. Software will guide analysis and data interpretation. Millipore will provide complete applications and product support
Smaller, stronger

Viability Index

Apoptosis Index

Cell Tracking

Absolute Cell Counting

Cell Cycle Analysis

Antigen Detection

GFP Expression

Immunophenotyping

Assay Outputs Delivered By The Guava Platform
The Power Of…
The Guava Benefits
Smaller, stronger – Guava flow cytometry

• Simply system: tiny footprint
  – Small sample volume, Easy sample preparation
  – No need for sheath fluid: 1/10~1/100 of conventional sample, 2,000-10,000 cells/Test
  – Less waste production: 1/100 of conventional waste

• System maintenance:
  – Fixed flow cell and cell flow adjust automatically
  – Easy to clear Clog
  – Easy to use daily QC tool: Guava Check Beads
  – No special operator need and realize personal
  – Low cost (No sheath fluid, micro-capillary, less waste, easy clean and less reagent)

• Stronger analysis software
  – High throughput
  – Use friendly software especially the software module
  – Compatible with different kinds of plate and EP
  – Absolute cell counting
  – Can characterize the percentage of Apoptotic cells
• Less training
• Less maintenance
• Less reagent
• Less running costs
• Less cells
• Less waste
• Less bench space

The Power of Small
The Power Of Choice

Many Assays. One Platform.

• Cell Counting/Viability- ViaCount
• Apoptosis
  – Mitochondrial Membrane Potential –
  – Caspase 3/7, Caspase 8, Caspase 9
  – Annexin V
  – Pan Caspases
  – TUNEL
• Cell Cycle
• Antigen Detection – up to 4 colors
• Immuno-Phenotyping- CD Marker Expression
• Fluorescent Proteins- GFP Expression
• Cytotoxicity
• Cell Tracking
• Cell Proliferation
• Antibody Quantitation- Rapid Quant
• Compound Screening-Secondary Screening
The Power Of…
On-Demand Cytometry
Grow Cells / Tissue Culture

Obtain cells from Library, Supplier or Organism

Stem Cells

Protein Expression

Media

Supplements

Cultureware

Sample Prep, Purify and Concentrate

Cell Analysis

Gene Transfer

Cell Adhesion

Cell Migration

Cellular Pathways

Cell Function

Focus of cell culture workflow module
- Epithelial CC
- Neural Stem Cell Culture

Focus of protein research workflow module

Cell based assay

SNAP i.d.
Outside of Cell

Guava
Inside of Cell

Luminex
HTS

Protein Analysis and Detection

Immunoprecipitation

Western Blot

IHC and ICC

Flow Cytometry

ELISA/RIA/Milliplex

Sample Prep, Purify and Concentrate

Cell Analysis

Gene Transfer

Cell Adhesion

Cell Migration

Cellular Pathways

Cell Function

Cell based assay

Focus of protein research workflow module

Cell based assay
Technical Leadership

Driven through key platforms

- SNAP i.d.™
  Western Blotting Solutions

- Milliplex Assays
  Multiplex Assays for Luminex

- Guava Flow Cytometry
  Instruments & Kits
  Benchtop Flow Cytometry

- Western Blotting 加速器
  - 18 kits
  - Leading metabolic disease & cytokines
  - Expanding offering in oncology

- 新一代毛细管
  - 技术分析仪
Sample Prep & Biotools

- Laboratory consumables used to handle and prepare samples in research work in many applications.
- These tools enable research by improving the productivity and robustness of scientific research and improve the quality of results.
- Key product lines:
  - Analytical sample prep devices
  - Protein purification and detection
  - Cell culture filtration
Drug Discovery Portfolios

• Research tools and services that support the discovery and development of new pharmaceuticals.

• Key product lines:
  – Screening products and services
  – Kinases/GPCR/Ion channel
  – Multiplex kits
    • Beadlyte
    • Lincoplex
  – Biomarker
  – Protein Detection Services
Millipore has a broad range of products that supports your research.

- Cell signaling
- Apoptosis
- Stem Cells
- Neuroscience
- Nuclear Function

- Antibodies
- Proteins
- Biotools
- Assay Kits
- Cell culture media & plates
Millipore Asia Tech Support Center
亚洲技术支持团队

亚洲技术服务信箱
asiatechserv@millipore.com

中国技术服务电话
400 889 1988

LS 全国经销商/销售培训中心

更专业、更快捷、更全面的中/英文服务
Thank You!