The Cells of the Immune System

Bone marrow

- pluripotent hematopoietic stem cell

- common lymphoid progenitor
- common myeloid progenitor
- granulocyte/macrophage progenitor
- megakaryocyte/erythrocyte progenitor

Blood

- B cell
- T cell
- neutrophil
- eosinophil
- basophil
- unknown precursor
- monocyte
- immature dendritic cell
- platelets
- erythrocyte

Granulocytes (or polymorphonuclear leukocytes)

Effector cells

- plasma cell
- activated T cell

Tissues

- mast cell
- macrophage
- immature dendritic cell

Lymph nodes

- mature dendritic cell
The Immune Reaction

- T helper cell (Th2) → B cells → Antibodies
- T helper cell (Th1) → Macrophages
- APC
- Antigen
- CD4^+
Applications for Monitoring the Immune Functions

- Use BrdU, Annexin V, and other methods to examine proliferation and apoptosis.
- Use optimized buffers and antibodies to look at transcription factor expression by flow cytometry.
- Measure phosphorylation status of key proteins with BD Phosflow antibodies.
- Use flow cytometry to sort cells or examine expression of cell surface markers.
- Examine cytokines expressed from a particular cell type with intracellular flow cytometry.
- Measure one secreted cytokine with ELISA or ELISPOT.
- Measure the levels of several cytokines simultaneously with BD CBA.
# Tools & Technologies for Immune Function Monitoring

<table>
<thead>
<tr>
<th>Tool/Technology</th>
<th>Flow Cytometry/Surface</th>
<th>Flow Cytometry/Intracellular</th>
<th>BD Cytometric Bead Array (CBA)</th>
<th>ELISPOT</th>
<th>ELISA</th>
<th>In Vivo Capture Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecules detected</td>
<td>Surface</td>
<td>intracellular and surface</td>
<td>Secreted or intracellular</td>
<td>Secreted (in situ)</td>
<td>Secreted</td>
<td>Secreted (in vivo)</td>
</tr>
<tr>
<td>Multiparameter</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Single cell subset information</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Frequencies, no subset information</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Antigen specific</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Post-assay viability</td>
<td>Yes</td>
<td>No</td>
<td>Yes, for secreted molecules</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Quantitation of protein</td>
<td>Possible*</td>
<td>Possible*</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Instrumentation</td>
<td>Flow cytometer</td>
<td>Flow cytometer</td>
<td>Flow cytometer</td>
<td>ELISPOT reader</td>
<td>Spectrophotometer</td>
<td>Spectrophotometer</td>
</tr>
</tbody>
</table>
Applications for Monitoring the Immune Functions – Surface & Intracellular

- Use BrdU, Annexin V, and other methods to examine proliferation and apoptosis.
- Use optimized buffers and antibodies to look at transcription factor expression by flow cytometry.
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Principle of Cell Surface Staining

Block Fc Receptors & Cell Surface Staining

Analysis by Flow Cytometry
Principle of Intracellular Cytokine Staining (ICS) Procedure

1. Stimulation
2. Block Protein Secretion
3. Block Fc Receptors & Cell Surface Staining
4. Intracellular Staining
5. Fixation & Permeabilization
6. Analysis by Flow Cytometry

- Anti-INF-γ
- CD69

wash
1. Selected Stimulation Methods

A. TCR mediated (CD3/CD28)
B. Mitogenic (PHA/Con A)
C. Polyclonal (PMA/Ionomycin)
D. Lipopolysacharride (LPS)
E. Superantigen (SEB)
F. Antigen specific (Peptides)
## 1. Selected Stimulation Methods

### Summary

<table>
<thead>
<tr>
<th>Method</th>
<th>Myeloid cell</th>
<th>B cell</th>
<th>T-helper cell</th>
<th>Cytotox T cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMA/Iono</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>PHA/ConA</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Very strong</td>
</tr>
<tr>
<td>LPS</td>
<td>Yes/ better + IFNγ</td>
<td>Yes</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>CD3/CD28</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Yes/ better + IL-2</td>
</tr>
<tr>
<td>IL-2</td>
<td>-</td>
<td>Yes</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Other cytokines</td>
<td>Normally weak/very individual -&gt; not possible to make general assumptions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEB + CD28/CD49d</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Very strong</td>
</tr>
</tbody>
</table>
1. Selected Stimulation Methods

Conclusion

Artificial activation

- Easy to perform
- In most cases strong cellular reaction in **ALL** susceptible cells
- **BUT**: No direct link to specific and natural processes
- Very good positive control **IN PARALLEL** to natural activations

Natural / specific activation

- Often quite difficult to perform perfectly well
- Often only weak signals
- Often only in very small **BUT** **VERY SPECIFIC** subpopulations
- **IN THE END** the only relevant system working with primary cells
2. Protein Transport Inhibitors

GolgiPlug (Brefeldin A) vs. GolgiStop (Monensin)

GolgiPlug™ inhibits secretion by disrupting the transport of cytokines from rough ER to Golgi complex.

GolgiStop™ inhibits secretion resulting in an accumulation of cytokines.
2. Protein Transport Inhibitors

• Cytokines are secreted fast upon expression

• Cytokine concentrations in cells are very low / not detectable

• Accumulation needed

• Substances are needed that delay cytokine secretion
  – Brefeldin A: BD GolgiPlug (cat# 555029): 1mg / ml in DMSO (1mM)
  – Monensin: BD GolgiStop (cat# 554724): 2mg / ml in EtOH (1mM)
Monensin (BD GolgiStop™) & Brefeldin A (BD GolgiPlug™) are commonly used to trap cytokines inside the cell for analysis.

- Monensin prevents protein secretion by interacting with the Golgi transmembrane Na\(^{++}\)/H\(^{+}\) transport.
- Brefeldin A redistributes intracellularly produced proteins from the cis/medial Golgi complex to the endoplasmic reticulum.
- Different inhibitors may work better for detection of different cytokines

<table>
<thead>
<tr>
<th>Species</th>
<th>Cytokines</th>
<th>Transport Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>IL-1(\alpha), IL-6, IL-8, TNF-(\alpha)</td>
<td>Monensin</td>
</tr>
<tr>
<td>Human</td>
<td>IFN-(\gamma), IL-2, IL-10, IL-12, MCP-1, MCP-3, MIG, MIP-1(\alpha), RANTES</td>
<td>Monensin or Brefeldin A</td>
</tr>
<tr>
<td>Mouse</td>
<td>IL-6, IL-12, TNF-(\alpha)</td>
<td>Brefeldin A</td>
</tr>
<tr>
<td>Mouse</td>
<td>GM-CSF, IL-3, IL-4, IL-5, IL-10</td>
<td>Monensin</td>
</tr>
<tr>
<td>Mouse</td>
<td>IFN-(\gamma), IL-2</td>
<td>Monensin or Brefeldin A</td>
</tr>
</tbody>
</table>
2. Protein Transport Inhibitors

Challenges

• Inhibition of intracellular protein transport by Monensin and Brefeldin A is toxic for cells

• Monensin and Brefeldin A do not only delay secretion of cytokines, but ALL protein secretion

• Maximum incubation time is 12-16hrs (protocol- and lab-dependent)

• Not all cytokines are expressed at high levels directly after activation

• Evaluate kinetics of cytokine expression for each activation method

• Perform activation and add Brefeldin A or Monensin only for the last 12hr
Effect of Protein Transport Inhibitors

No GolgiStop  With GolgiStop

- IL-2 (PE)
- TNF-α (PE)
- IFN-γ (PE)
3. Blocking of Unspecific Staining

- **Mouse and Rat Systems**
  - BD FcBlock™ (anti-CD32/CD64)

- **Human Systems**
  - Excess of irrelevant purified Ig
  - Human serum
4. Cell Surface Antigen Staining

Diagram showing the process of staining cell-surface antigens.

- Unlabeled Cell Surface antigens
- Stained Cell-Surface Antigens
5. Cell Fixation and Permeabilization

Fix prior to permeabilization to maintain structural integrity

Fix = Paraformaldehyde
Permeabilize = Saponin
6. Controls for ICS

**Purified Antibody**
- Pretreatment of cells with purified antibody from the same hybridoma clone blocks the binding of the fluorescent antibody to the ligand.

**Recombinant Cytokine**
- Excess recombinant cytokine prevents binding of fluorescent anti-cytokine antibodies.

**Isotype Control**
- Non-specific binding of fluorescent Ig isotype controls.
6. Controls for ICS

- Demonstrates inherent “non-specific” background levels
- Negative staining control
- Subtract “non-specific” staining from positive cytokine staining, these are actual positives
6. Controls for ICS
Control Cells

• **Fixed cytokine positive cells**
  - Staining control
  - Workflow control
  - Antibody titration

• **Human**
  - HiCK-1, HiCK-2, HiCK-3, HiCK-4

• **Mouse**
  - MiCK-1 (IL-2, IFN-γ, TNF-α)
  - MiCK-2 (IL-3, IL-4, IL-10, GM-CSF)

Representative expression of IL-2 (A: PE-MIQ1-17H12, Cat. No.18955A), IFN-γ (B: PE-4S.B3, Cat. No. 18505A), and TNF-α, (C: PE-MAb11, Cat. No.18645A) and light-scattering characteristics, (D) by HiCK-1 Positive Control Cells.
Detection & Quantitation of Soluble Proteins, Transcription Factors & Phospho-Proteins

**ELISA**

Use BrdU, Annexin V, and other methods to examine proliferation and apoptosis.

Use optimized buffers and antibodies to look at transcription factor expression by flow cytometry.

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Measure phosphorylation status of key proteins with BD Phosflow antibodies.

Examine cytokines expressed from a particular cell type with intracellular flow cytometry.

Measure the levels of several cytokines simultaneously with BD CBA.

Measure one secreted cytokine with ELISA or ELISPOT.
Principle of an ELISA

**ELISA: Enzyme Linked Immunosorbent Assay**

1. Capture Antibody, Incubate overnight
2. Wash, Block Plates, Incubate 1 hr. RT
3. Wash, Add Standard/Sample, Incubate 2 hr. RT
4. Wash, Add Detection Antibody, Incubate 1 hr. RT
5. Wash, add Substrate Incubate 30 min. RT
6. Add Stop Solution Read at 450 nm
Principle of an ELISA
ELISA Technology Features

- Measures soluble proteins in supernatants and lysates
- Quantitative results
- Highly sensitive compared to single Ab applications
- Fast results
- No single cell analysis
Detection & Quantitation of Soluble Proteins, Transcription Factors & Phospho-Proteins

CBA

Use BrdU, Annexin V, and other methods to examine proliferation and apoptosis.

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Measure the levels of several cytokines simultaneously with BD CBA.
CBA - Definition

- Fluorescent immuno-assay allowing the simultaneous detection and quantification of proteins within one sample using flow cytometry

- CBA:
  - Cytometric: Data acquired on a flow cytometer is analysed using specific software
  - Bead: The system is composed of various beads with different fluorescence intensity coated with high affinity capture antibodies
  - Array: Technology allowing multiplex analysis
The Cytometric Bead Array (CBA)

Beads provide an expandable assay platform for use with a flow cytometer

- Multiple sizes
- Different intensities
- Different colors with different intensities

CBA Kits
CBA Flex Sets
Enhanced Sensitivity Flex Sets
How does a CBA Assay work?

Capture Ab + Capture Bead

Analyte of Interest + Fluorescent Detector Ab
CBA Basics

1. Prepare samples
2. Acquire samples
3. Analyze samples
Principle of BD™ CBA Kits

One Step

3 h incubation
BD™ CBA Kits

Cytokine standard
Detection-antibodies
Assay Buffer
Capture-Beads
Wash buffer
Instrument calibration
## BD™ CBA Kits

### Human Portfolio

<table>
<thead>
<tr>
<th>Kit content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaphylatoxin Kit C4a, C3a, C5a</td>
</tr>
<tr>
<td>Chemokine Kit IL-8, RANTES, MIG, MCP-1, IP-10</td>
</tr>
<tr>
<td>Inflammation Kit IL-8, IL-1b, IL-6, IL-10, TNF, IL-12p70</td>
</tr>
<tr>
<td>Th1/Th2 Cytokine Kit IL-2, IL-4, IL-5, IL-10, TNF, IFNg</td>
</tr>
<tr>
<td>Th1/Th2 Cytokine Kit II IL-2, IL-4, IL-6, IL-10, TNF, IFNg</td>
</tr>
</tbody>
</table>

### Mouse Portfolio

<table>
<thead>
<tr>
<th>Kit content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoglobulin Isotyping Kit IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, IgE</td>
</tr>
<tr>
<td>Inflammation Kit IL-6, IL-10, MCP-1, IFNg, TNF, IL-12p70</td>
</tr>
<tr>
<td>Th1/Th2 Cytokine Kit IL-2, IL-4, IL-5, TNF, IFNg</td>
</tr>
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</table>

### Non-Human Primate

<table>
<thead>
<tr>
<th>Kit content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1/Th2 Cytokine Kit IL-2, IL-4, IL-5, IL-6, TNF, IFNg</td>
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</table>

### Standards

<table>
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<tr>
<th>Kit content</th>
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</thead>
<tbody>
<tr>
<td>Human Inflammation Standard IL-8, IL-1b, IL-6, IL-10, TNF, IL-12p70</td>
</tr>
<tr>
<td>Human Th1/Th2 Cytokine Standard IL-2, IL-4, IL-5, IL-6, IL-10, TNF, IFNg</td>
</tr>
<tr>
<td>Mouse Inflammation Standard IL-6, IL-10, MCP-1, IFNg, TNF, IL-12p70</td>
</tr>
<tr>
<td>Mouse Th1/Th2 Cytokine Standard IL-2, IL-4, IL-5, TNF, IFNg</td>
</tr>
</tbody>
</table>
BD™ CBA Kits

- Preconfigured kits for consistent results with routine panels
- Available for functional areas of biology such as Th1, Th2, Th17, inflammatory cytokines, anaphylatoxins
- Measure up to seven analytes simultaneously
- Compatible with flow cytometers that have a 488nm laser
Principle of BD CBA Flex Sets

CBA Soluble Protein Flex Set

- Beads in bead diluent w/o colors
- Beads + Sample/standard with assay diluent yellow
- Total 2-3 hours

CBA Cell Signaling Flex Set (cell lysate)

- Beads + Sample/standard with detector diluent = green
- Beads + Sample/standard with assay diluent yellow
- Total 4 hours

1 hour + 1 or 2 hours
# CBA Kits vs. CBA Flex Sets

<table>
<thead>
<tr>
<th></th>
<th>CBA</th>
<th>CBA Flex</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of beads</strong></td>
<td>6-7</td>
<td>30</td>
</tr>
<tr>
<td><strong>Bead fluorescence</strong></td>
<td>FL-3 (670nm) or FL-4 (660nm)</td>
<td>FL-3 (670 or 785)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FL-4 (660)</td>
</tr>
<tr>
<td><strong>Detection method</strong></td>
<td>PE</td>
<td>PE</td>
</tr>
<tr>
<td><strong>Sample</strong></td>
<td>In solution</td>
<td>In solution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cell lysate ; Cell Signaling</td>
</tr>
<tr>
<td><strong>Flow cytometer</strong></td>
<td>1 laser 488nm</td>
<td>2 lasers : 488 &amp; 635</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or 532 &amp; 635</td>
</tr>
<tr>
<td><strong>Software</strong></td>
<td>FCAP</td>
<td>FCAP</td>
</tr>
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</table>
CBA Flex Sets: Bead Identification

30 bead positions available

<table>
<thead>
<tr>
<th>Cluster ID</th>
<th>Cluster ID</th>
<th>Bead</th>
<th>Bead</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Hu IL-1bet</td>
<td>BD-dA4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Hu IL-2</td>
<td>BD-dA5</td>
<td></td>
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<tr>
<td>3</td>
<td>Hu IL-4</td>
<td>BD-dA7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Hu IL-5</td>
<td>BD-dA8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Hu IL-6</td>
<td>BD-dA9</td>
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<tr>
<td>6</td>
<td>Hu IL-7</td>
<td>BD-dB4</td>
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<td>7</td>
<td>Hu IL-8</td>
<td>BD-dB5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Hu IL-10</td>
<td>BD-dB7</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Hu IL-12</td>
<td>BD-dB8</td>
<td></td>
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<tr>
<td>10</td>
<td>bFGF</td>
<td>BD-dC5</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>FAS-L</td>
<td>BD-dC6</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>G-CSF</td>
<td>BD-dC8</td>
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<tr>
<td>13</td>
<td>GM-CSF</td>
<td>BD-dC9</td>
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<tr>
<td>14</td>
<td>TNF</td>
<td>BD-dD4</td>
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</tr>
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<td>15</td>
<td>IP10</td>
<td>BD-dD5</td>
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<td>16</td>
<td>LT-alpha</td>
<td>BD-dD7</td>
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<tr>
<td>17</td>
<td>MCP-1</td>
<td>BD-dD8</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>MIF</td>
<td>BD-dD9</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>MIF-Talpha</td>
<td>BD-dE4</td>
<td></td>
</tr>
</tbody>
</table>
100 tests, packed as “set”

- Capture beads
- Detector reagents
- Standard (x2)
- Contains a simple TDS with performance info
BD™ CBA Flex Master Buffer Kit

100 and 500 test sizes

• includes all buffers and setup reagents
  • Mouse/Rat
  • Human
  • Cell Signaling
• Contains a Manual with setup instructions and assay protocol details
<table>
<thead>
<tr>
<th>CBA Kits</th>
<th>CBA Flex Sets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Signaling Flex Sets</td>
</tr>
<tr>
<td>contained in the kit</td>
<td>Cell Signaling Master Buffer Kit (for 500 or 100 tests)</td>
</tr>
<tr>
<td></td>
<td>Mouse/Rat Soluble Protein Master Buffer Kit (for 500 or 100 tests)</td>
</tr>
</tbody>
</table>

The different types of flex sets cannot be mixed and performed in the same sample.
BD™ CBA Flex Portfolio

- Soluble Proteins – Human (e.g. G-CSF, GM-CSF, IFNγ, IL-1β, IL-2, IL-3,...)
- Soluble Proteins – Immunoglobulins (e.g. Total IgG, IgG1, IgG2,...)
- Phospho-specific CBA Flex Sets (e.g. Akt1, Akt2, Akt3, ERK1/2,...)
- Soluble Proteins – Mouse (e.g. IFNγ, IL-1α, IL-2, IL-3, IL-4, IL-5,...)
- Soluble Proteins – Rat (e.g. IFNγ, IL-4, IL-6, IL-10, TNF)
- Soluble Proteins – Supporting Reagents (e.g. Hu / Ms Master Buffer Kit,...)
- Soluble Proteins – Standards (e.g. IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8,...)
- Functional Beads (positions A4 – E9, own specificities)
- Functional Beads – Supporting Reagents (buffer, IgG detector ab)
**ES Flex Sets vs. Classical Flex Sets**

**Classic Soluble CBA Flex**
- Capture 1 hour
- Detection 1 or 2 hours
- Total 2-3 hours

**Enhanced Sensitivity CBA Flex**
- Capture 2 hours
- Detection 3 hours
- Total 5 hours
BD CBA Enhanced Sensitivity Flex Sets

- **A new family of Flex Sets**
  - Assay range:

<table>
<thead>
<tr>
<th>Classic CBA Flex Set</th>
<th>Enhanced Sensitivity Flex Set</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 - 2500 pg/ml</td>
<td>0.274 – 200 pg/ml</td>
</tr>
</tbody>
</table>

- **Allows cytokine detection in highly diluted samples**
- **Based on established CBA antibody pairs**
- **Tested in supernatant, serum, and plasma samples**
  - Main advantage in supernatant samples: can detect at earlier time points - that were previously below the level of detection
  - “Normal” serum and plasma samples still have undetectable amounts of analyte
BD CBA Enhanced Sensitivity Flex Sets

- **Flex Set (100 Tests)**
  - One vial of capture beads
  - One vial of detection reagent (Part A)
  - Two vials of standard

- **Master Buffer Kit (100 test and 500 test)**
  - Contains all buffers and set-up reagents
    - No Bead Diluent for Serum/Plasma
    - Contains more wash buffer
  - Contains the Enhanced Sensitivity Detection Reagent (Part B)
ES Flex Sets Can Detect Analytes Earlier in a Time Course

Human PBMC stimulated with PMA/Ionomycin

**Note:** No data reported when falling below the last standard curve point

**Legend:**
- Red: Flex Set
- Blue: ES Flex Set
<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay range of 274 – 200,000 fg/mL</td>
<td>Can detect early points in a time course and highly diluted samples</td>
</tr>
<tr>
<td></td>
<td>35 fold sensitivity improvement as compared to classic CBA</td>
</tr>
<tr>
<td>Each ES CBA flex set has a different bead position</td>
<td>A fully flexible and cost-effective multiplex panel as compared to kits that may include inappropriate cytokines</td>
</tr>
<tr>
<td></td>
<td>Analyzes up to 10 soluble proteins with just 25 to 50 μL of sample</td>
</tr>
<tr>
<td>Based on existing Flex Set technology</td>
<td>Fast and easy implementation</td>
</tr>
</tbody>
</table>
Acquisition of Samples

The BD Accuri C6 Flow Cytometer System

An affordable, full-featured, easy-to-use flow cytometer.
Two lasers and six detectors
Analysis of Data

• FCAP Array v3.0 Analysis Software
  – Compatible with FCS 2.0 or 3.0 files from any flow cytometer
  – Integrated workflow for the BD FACSVerse flow cytometer
  – Windows® 7, Vista®, or XP compatible
Accuri C6 & CBA Summary

- A perfect and complete solution to measure & quantify multiple proteins simultaneously
  - CBA Flex Sets & Kits
    - Broad spectrum of specificities, ready-to-use or full flexibility

- BD Accuri C6 with C6Sampler option
  - Can be also used for other flow cytometric analysis
  - Specific templates available for acquisition & analysis of CBA Kits and Flex Sets

- FCAP Array software

- Application and technical support
Feature of CBA Products

• Up to 30 proteins detectable in parallel
  - Pre-configured kits or individual sets to mix and match your demands

• Sensitivity limit as low as 0.274 pg/mL

• Only 25 to 50 µL of sample needed
  - Multiple quantitative results from a single small-volume sample

• Significantly reduced time to results
  - High-performance optimized reagents for shortest time-to-results
  - No assay formulation required, regardless of plex size
  - Automated sample acquisition and increased throughput options with 96-well plate analysis

• A complete solution
  - Broad instrument compatibility and dedicated analysis software

BD
Applications for Monitoring the Immune Functions

- Use BrdU, Annexin V, and other methods to examine proliferation and apoptosis.
- Use optimized buffers and antibodies to look at transcription factor expression by flow cytometry.
- Use flow cytometry to sort cells or examine expression of cell surface markers.
- Measure phosphorylation status of key proteins with BD Phosflow antibodies.
- Examine cytokines expressed from a particular cell type with intracellular flow cytometry.
- Measure one secreted cytokine with ELISA or ELISPOT.
- Measure the levels of several cytokines simultaneously with BD CBA.