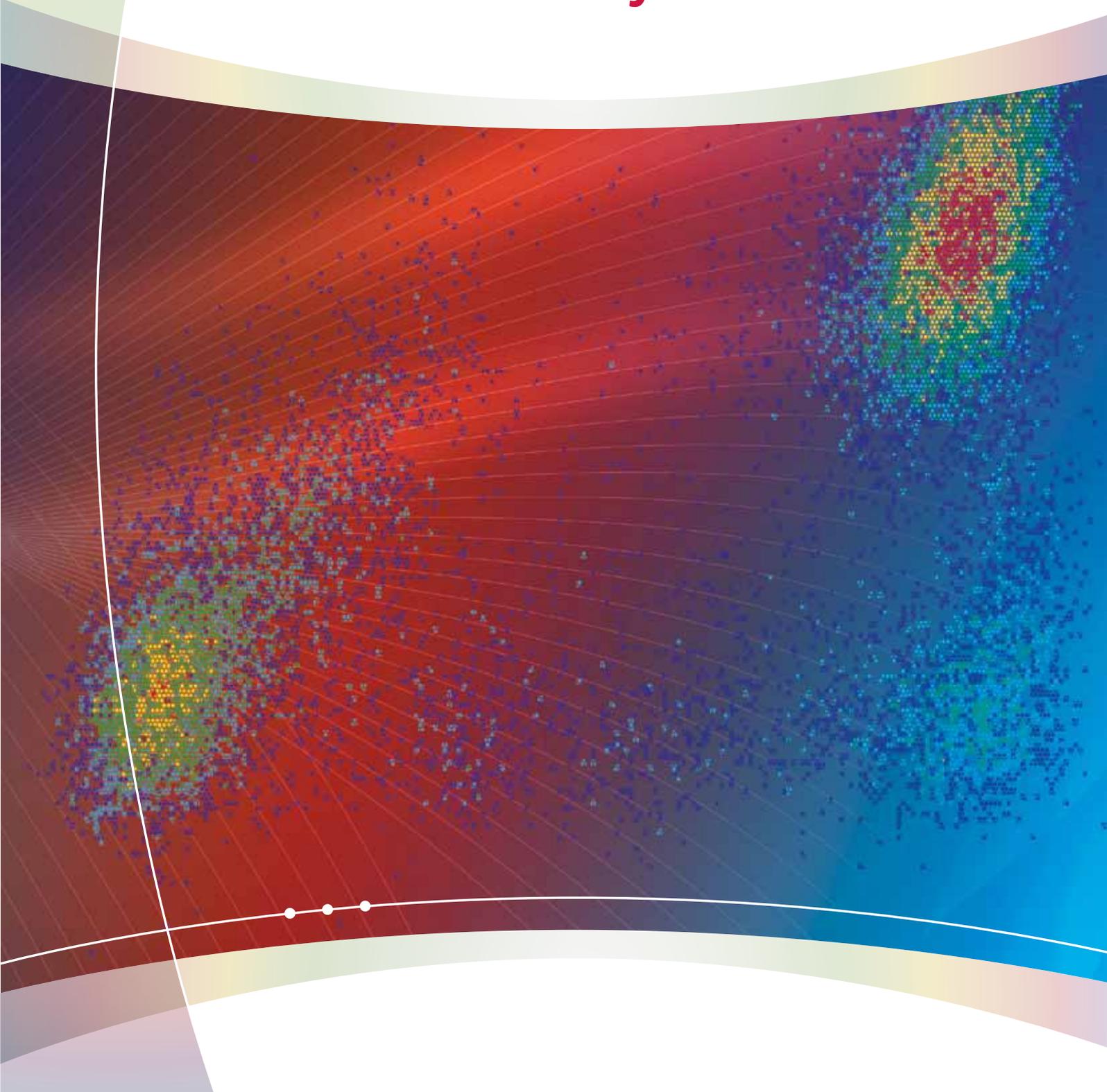


Custom Reagents & Contract Assay Services



eBioscience, an Affymetrix company, is committed to developing and manufacturing high-quality, innovative reagents using an ISO certified process. As a provider of more than 11,000 products, we empower our customers worldwide to obtain exceptional results by using reagents that offer a new standard of excellence in the areas of innovation, quality and value.

Unless indicated otherwise, all products are For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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eFluor® is a registered trademark of eBioscience, Inc.

Alexa Fluor®, Pacific Blue® and Texas Red® are registered trademarks of, and licensed under patents assigned to Molecular Probes, Inc. (Life Technologies).

Cyanine (Cy) dye conjugates are covered by US Patent Nos. 4,981,977 and 5,268,486. Cy™, including Cy™5, Cy™5.5 and Cy™7, are trademarks of Amersham Biosciences Ltd. (GE Healthcare)

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Custom Products & Services

eBioscience Custom Group offers custom reagent manufacturing and contract assay development services to help expedite your research. The eBioscience team has the expertise to provide comprehensive solutions your research requires for:

- Bulk antibodies and proteins
- Custom conjugations
- Immunophenotyping
- Immunoassay development
- Procarta™ custom multiplex assays
- Contract manufacturing

Our Commitment to Quality

eBioscience is committed to developing and manufacturing high-quality, innovative reagents in an ISO-certified facility. As a provider of more than 11,000 products, we empower our customers worldwide to obtain exceptional results by using reagents that offer a new standard of excellence in the areas of innovation, quality and value.

Trusted. Cited. Reliable.

- Established in 1999
- Cited in more than 30,000 scientific articles
- ISO 13485:2003/AC2009-certified quality system
- Extensive FDA registered Analyte Specific Reagent (ASR) portfolio
- Developer of more than 600 immunoassays
- CE-IVD marked reagents & ELISA kits

Bulk Reagents

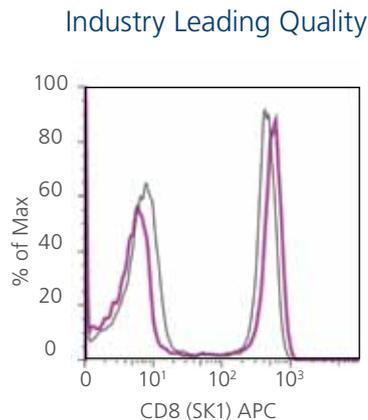
eBioscience offers more than 11,000 reagents used every day in research and clinical applications which are available for bulk volume purchase. Bulk volume reagents guarantee absolute consistency. By removing potential lot-to-lot variability, bulk reagents are perfect for long-term studies. Our in-house manufacturing capabilities can support even the largest project requirements. When you require a customized packaging option, eBioscience will deliver a solution that best fits your workflow.

Consider a bulk purchase when you require:

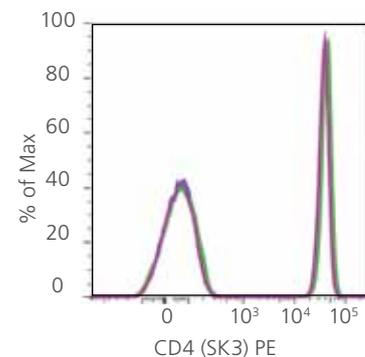
- Cost-effective purchases
- Design consultation and project assistance
- Timely delivery
- Lot-to-lot consistency
- Custom formulations and packaging flexibility
- Customer-defined concentrations
- Complete quality documentation
- Quality-controlled ISO manufacturing

Delivering Performance

(LEFT) eBioscience antibody conjugates for flow cytometry meet or exceed the performance standards in the industry. Shown is a representative histogram overlay of human PBMCs stained with the same clone and format manufactured by eBioscience (purple histogram) or an alternative supplier (gray histogram).



Lot-to-lot Consistency



Manufacturing Consistency

(RIGHT) Consistent performance between production lots is a requirement of the eBioscience manufacturing specifications. Pictured here is a representative histogram overlay of human PBMCs prepared from a single donor and stained with the same antibody clone and format representing three independent production runs. Each lot is represented by a different color (purple, blue, or green) histogram.

Functional Reagents for Cell-based Assays

An intrinsic part of evaluating any biological system is the ability to mimic and/or inhibit relevant pathways, both at the cellular level and in tissue and whole-organism models. The use of bioassays has allowed life science researchers in every field to explore these biologic systems with consistent, reproducible results and to systematically examine:

- Initiation of cell signaling via receptor-ligand interactions
- Induction of intracellular pathways that promote cell activation and differentiation
- Expression of unique phenotypes
- Protein expression patterns

eBioscience offers an expansive catalog of functional reagents providing optimal results when used in activation, neutralization or blocking studies.

In-house manufacturing expertise and flexibility enables us to provide custom functional grade solutions for almost any antibody clone or recombinant protein available in our catalog.

Functional Grade proteins and antibodies:

- Provided in appropriate buffers
- Contain endotoxin levels significantly lower than the industry standard
- Always sodium azide-free for *in vivo* or *in vitro* applications
- Conjugation ready

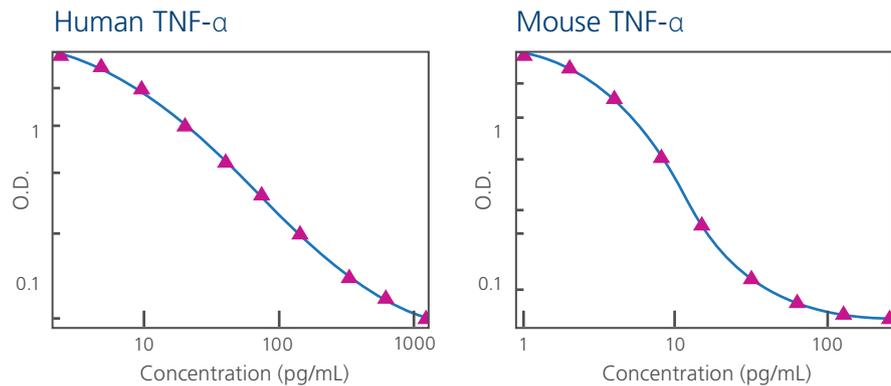
Antibodies	Proteins
Choose our standard quality criteria	
>90% purity and <10% aggregation (by HPLC)	>95% purity
<0.001 ng/μg antibody endotoxin levels	<0.01 ng/μg protein endotoxin levels
Azide-free formulation	Azide-free formulation
Conjugation-ready	Carrier-free formats are available
Define your own	
Custom formulations and packaging available	
Special bulk pricing	
Single-lot capabilities	
Customer-defined concentrations	

Recombinant Proteins

eBioscience has best-in-class recombinant proteins for cell culture maintenance, proliferation and/or cell differentiation. A number of cytokines and chemokines are found in our ever-expanding catalog for inducing growth, stimulating and other factors. All our proteins are available in multiple formats, including carrier-free options that surpass the industry-standard endotoxin specifications for *in vivo* or *in vitro* applications.

TNF- α Cytotoxicity

When used *in vitro*, human recombinant (cat. no. 34-8329) and mouse recombinant (cat. no. 34-8321) TNF- α are biologically active and induce cytolysis of mouse L929 cells, a standard assay to assess cytotoxic activity of these proteins.

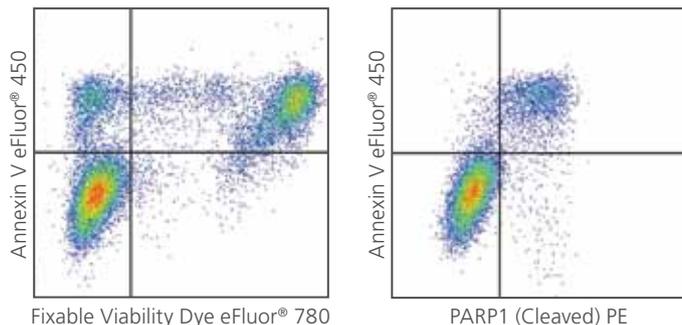


Functional Grade Antibodies

The term “functional grade antibody” refers to those antibodies that either mimic or interrupt the natural biologic effects associated with ligand-receptor interactions. eBioscience offers Functional Grade antibodies which may be used for activation, neutralization, and/or blocking studies. These reagents are provided in appropriate buffers containing extremely low endotoxin levels, and are sodium azide free for use *in vivo* or *in vitro* applications.

Anti-Human CD95 (Fas) clone EOS 9.1, induces apoptosis in human cells

Jurkat cells were stimulated with Anti-Human CD95 (cat. no. 16-0958) Functional Grade Purified. Stimulated cells were then harvested and stained sequentially with Fixable Viability Dye eFluor[®]780 (cat. no. 65-0865) and Annexin V Apoptosis Detection Kit eFluor[®] 450 (cat. no. 88-8006), followed by fixation / permeabilization using Foxp3 Fixation/Permeabilization Buffer (cat. no. 00-5521) and intracellular staining with Anti-Human PARP1 (Cleaved) PE (cat. no. 12-6668). Total cells (top) or viable cells (bottom) were used for analysis.



Functional Grade Antibody Performance: Quality First.

Quality and value are paramount when choosing reagents for your studies. At eBioscience, we manufacture our products to meet stringent performance specifications, so you can feel confident that a new lot of antibody will perform consistently with previous lots.

Functional Grade Purified Antibodies

- SDS-PAGE and HPLC testing ensures the purity and integrity of the antibody
- Flow cytometry ensures functionality of the antibody
- LAL tests determine the level of endotoxin
- Sterility test

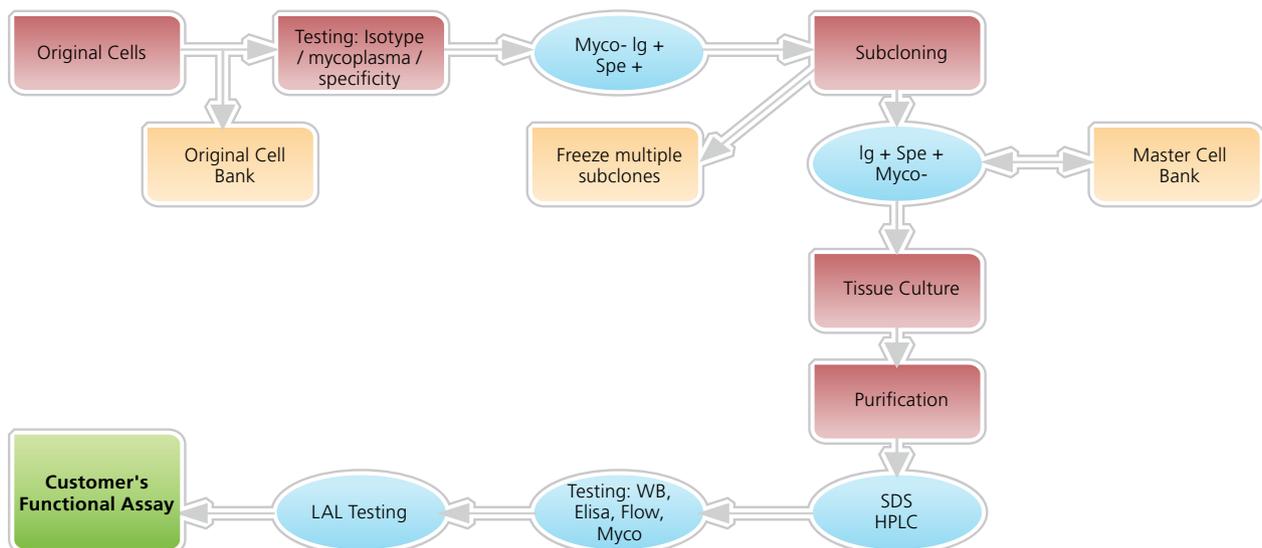
Cell Banks

- Cell lines are tested for specificity, mycoplasma and isotype
- Cell lines go through subcloning to ensure clonality of the produced antibody
- During production master cell lines are re-tested for specificity, mycoplasma and isotype integrity

Cell Culture

- Functional Grade purified antibodies are expressed in tissue culture, never through ascites, to reduce the risk of contaminants and non-specific antibodies.

Antibody Manufacturing Process



Custom Antibody Conjugation & Formulation

Formats and Formulations

Whether you require specialized fluorophore pairings or custom formulations and packaging, our state-of-the-art facility in San Diego, California, is poised to provide an affordable solution. eBioscience is an expert in antibody conjugation, multicolor panels and multicolor cocktails.

Full-Spectrum Fluorophore Options

- More than 15 different formats
- Choose from our catalog of antibodies or provide your own
- Non-fluorophore options:
 - Biotin
 - Alkaline phosphatase (AP)
 - Horseradish peroxidase (HRP)

Specialized Formulations and Concentrations

- Options for *in vitro* or *in vivo* assays
- New industry “gold standard” for endotoxin levels (<0.001 ng/ μg which exceeds industry standard of 0.01 ng/ μg)
- Large variety of buffers to support your applications

Fluorophores

Alexa Fluor® 488

Alexa Fluor® 532

Alexa Fluor® 647

Alexa Fluor® 700

APC

APC-eFluor® 780

eFluor® 450

eFluor® 570

eFluor® 615

eFluor® 660

eFluor® 710

FITC

PE

PE-Cy5

PE-Cy5.5

PE-Cy7

PerCP

PerCP-Cy5.5

PerCP-eFluor® 710

eFluor® Fluorophores and Dyes

eFluor is the eBioscience brand of fluorophores and dyes developed specifically for labeling and detection of biomolecules. Within the eFluor brand you will find organic dye fluorophores for cell analysis and fluorescent imaging, as well as functional dyes that enable cell proliferation and cell viability assays. Each eFluor fluorophore is engineered for superior optical performance in a variety of applications.

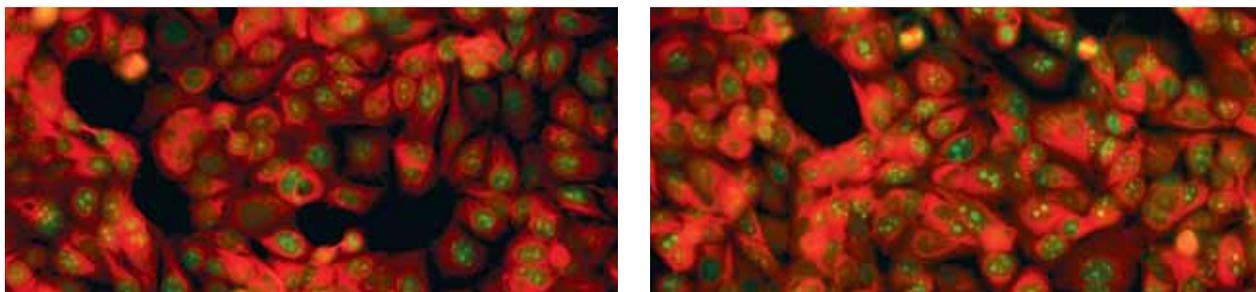
eFluor® Organic Dyes

- Direct conjugates for flow cytometry
- Superior optical performance in multicolor flow cytometry and immunohistochemistry (IHC)
- Fully compatible with other organic or protein-based dyes

eFluor® Functional Dyes

- Small-molecule organic dyes
- Viability dyes for intracellular staining
- Proliferation dyes for tracking cell division
- Dyes for monitoring intracellular-free calcium

eFluor® 615 – Designed for fluorescence imaging; offering excellent resolution and photostability



Comparison of Anti-alpha Tubulin eFluor® 615 and Anti-alpha Tubulin Alexa Fluor® 594

MCF-7 cells fixed, permeabilized and stained with Anti-Human Ki-67 FITC (cat. no. 11-5699) and Anti-alpha Tubulin eFluor® 615 (left, 342ms; cat. no. 42-4502) or Anti-alpha Tubulin Alexa Fluor® 594 (right, 119ms). When using eFluor® 615 antibody conjugates, we recommend a filter set that will capture the 615 emission wavelength (for example, Excitation 560/55, 585LP, Emission 645/75). A standard Alexa Fluor® 594 filter set is acceptable.

Custom Immunophenotyping with Antibody Cocktails

3-Phase Process

1. Consult

- Project reviewed with client
- Performance criteria established
- Project start and end dates defined

2. Develop & Validate

- Determine optimal clones and formats
- Formulate, test, optimize and standardize a cocktail using defined cells or tissues
- Validate performance of the cocktail
- Integrate accelerated stability testing

3. Manufacture

- Scale, manufacture, package and deliver under ISO-certified processes

Flowcytometry is an indispensable technique for deciphering complex cellular processes and interactions in a variety of systems that model normal and disease states. The development of cytometers equipped with 3-5 lasers and capable of detecting >15 fluorescent parameters makes flow cytometry a fundamental tool for the life scientist. Realizing the power of multicolor flow cytometry requires high performance fluorochromes paired with appropriate specificities to ensure consistent and reliable data.

eBioscience specializes in developing and manufacturing antibody "cocktail" panels that meet your exact experimental requirements.

Benefits of Creating Custom Cocktails

Standardization

- Correlate data globally
- Streamline sample preparation
- Option to include compensation controls, buffers and protocols
- Improve reproducibility
 - Technician-to-technician variability
 - Lab-to-lab (site-to-site) variability
 - Experiment-to-experiment variability

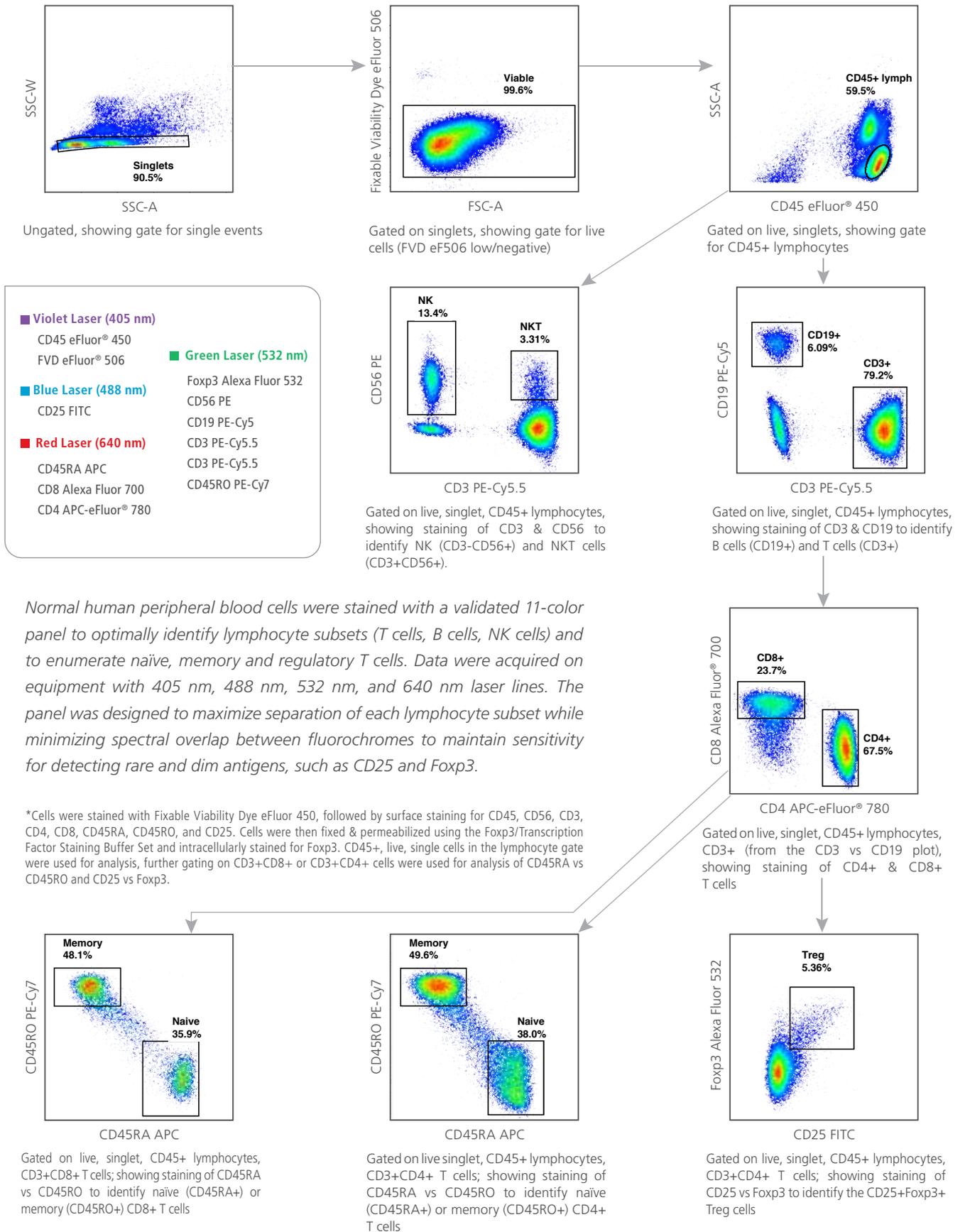
Quality

- Establish control by setting release criteria
- Manufactured and quality controlled under our ISO-certified processes
- Reduce risk and remove variables

Efficiency

- Reduce the number of individual antibody purchases improving inventory and purchasing control
- Test-size units available for purchase
- Eliminate redundant development, optimization and validation for the same panel by multiple groups

Example of 11-Color Flow Cytometry Panel



Immunoassay Development & Validation

Immunoassay development begins with an in-depth consultation with our scientific team. Project feasibility is determined based on the availability of assay components, compatibility of reagents with the desired assay and other project-specific parameters. eBioscience can also assist with reviewing publications, sourcing reagents and forming third-party collaborations.



Three-Phase Development

1. Feasibility and Proof of Concept

- Assay design: immunoassay format, sample matrix, sensitivity and dynamic range
- Identify reagents required for optimal results
- Preliminarily define assay characteristics and performance



2. Developmental Phase

- Establish assay parameters, e.g. limit of detection
- Define working range and sensitivity
- Evaluate matrix effects and assay reproducibility



3. Assay Validation and Tech Transfer

- Assess reagent stability and robustness
- Measure inter- and intra-assay reproducibility, accuracy and specificity
- Sample screening and analysis platform compatibility
- Scale up and transfer to high-throughput stages

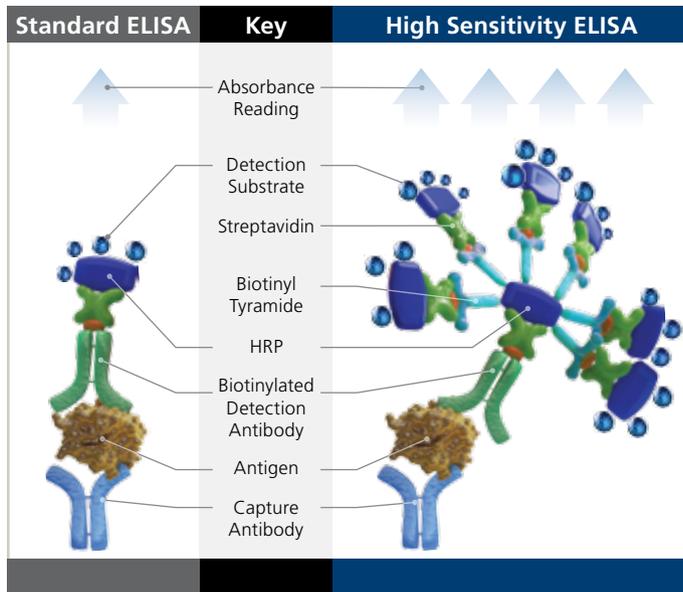
Customizable Immunoassay Platforms

Platinum ELISA Kits – Platinum ELISA kits are standard sandwich immunoassays.

Instant ELISA® – Instant ELISA technology represents a major improvement of the established sandwich ELISA. The name “instant” says it all: adding the sample is all that is needed to start the assay. There is no laborious preparation of reagents, serial dilution of standards or their sequential addition to the plate. In contrast to conventional ELISA protocols, the Instant ELISA plate contains coating antibody and lyophilized detection antibody, streptavidin-HRP and sample diluent. Additional wells containing the ready-to-use standard curve are provided separately.

High-Sensitivity ELISA Kits – High Sensitivity ELISAs provide a solution for difficult-to-detect, low-expressing proteins from serum, plasma or cell culture samples. Improved sensitivity is made possible by the inherent design of further enhancing the signal obtained from conventional HRP-based ELISA. The signal amplification step, using biotinylated Tyramid, results in an increased sensitivity without loss of resolution or increase in background.

- **Sensitive** – Detect low cytokine concentrations ≤ 1.0 pg/mL for reliable quantification
- **Low Sample Volume** – 50 μ L sample volume required. Ideal for limited sample resources
- **Use Existing Instrumentation** – Standard colorimetric plate reader with absorption measurement at 450 nm



High Sensitivity ELISA Solution

The amount of reacted biotinyl-Tyramide is proportional to the amount of HRP in the well.

Following incubation, unbound biotinylated Tyramide is removed during a wash step.

After removal of excess biotin-Tyramide from the wells, streptavidin-HRP (Amplification Reagent II) is added, and binds to the additional biotin sites created during the biotin-Tyramide reaction. This amplification step effectively multiplies the quantity of HRP molecules for the final TMB substrate reaction.

Procarta™

Custom Configured Multiplex Immunoassays*

Multiplex Immunoassays

Human

Cytokine Panel 1 (54 plex)
 Cytokine Panel 2 (36 plex)
 MMP Panel 1 (5 plex)
 MMP Panel 2 (3 plex)
 Coagulation Panel
 Apolipoprotein Panel
 Single Plex Assays

Mouse

Cytokine Panel 1 (37 plex)
 Cytokine Panel 2 (7 plex)
 Antibody Isotyping Panel
 Single Plex Assays
 IFN alpha/beta

Non-Human Primate

Cytokine Panel 1 (45 plex)
 Single Plex Assays

Rat

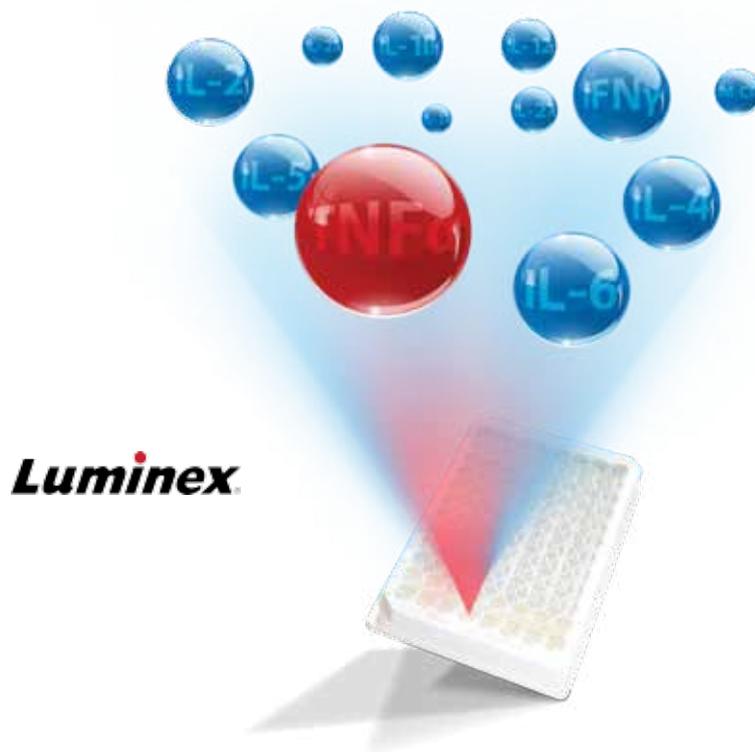
Cytokine Panel 1 (30 plex)
 Single Plex Assays

Canine

Cytokine Panel 1 (12 plex)
 Single Plex Assays

Porcine

Cytokine Panel 1 (10 plex)
 Single Plex Assays



Use the Platform of Choice to Customize Your Biomarker Profile

Procarta immunoassays are quantitative multiplex immunoassays using Luminex® xMAP® technology for the biomarker profiling of up to 100 analytes in a single 25 µL sample. The Procarta immunoassay portfolio supports the top biomarker (and many other) targets offering over 300 analytes across six species. The largest commercially available Luminex cytokine panel—the Human Cytokine Panel 1 54 plex—can now be found at eBioscience.

Unparalleled Flexibility and Expandability

Procarta multiplex immunoassays are custom blended and optimized to deliver superior results tailored to the panel design of YOUR choice.

For your customized mix & match multiplex panel, simply:

- Choose the analytes from each of the panels required for your research
- Select the bead type (magnetic or polystyrene bead formats)
- Let us know the sample type and instrument used

Visit www.eBioscience.com/application/multiplexing.htm to learn more about the Luminex xMAP Technology and the options available.

*Not all products available in all regions. Please contact customer service for more information.

Procarta™ Immunoassay Workflow

Figure 1

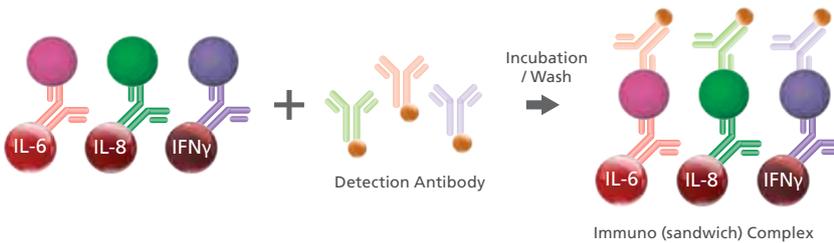


Capture Target Analytes

- Analyte-specific capture beads are added to prepared sample. The unique, differentially dyed capture beads are coated with target-specific capture antibodies. This allows for the unique identification of the analyte.

Incubate 1 hour and wash

Figure 2

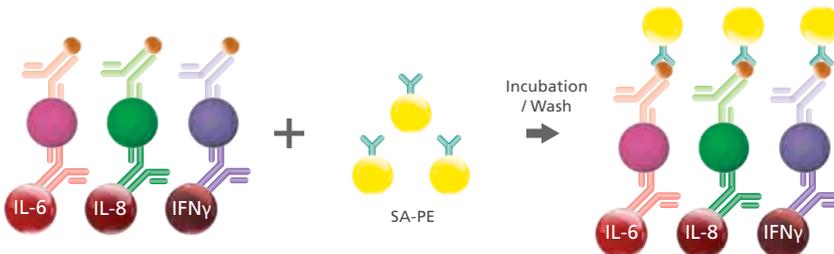


Detect Captured Analyte

- Captured bead/analytes are incubated with biotinylated analyte-specific detection antibody to form an immuno (sandwich) complex.

Incubate 1 hour and wash

Figure 3

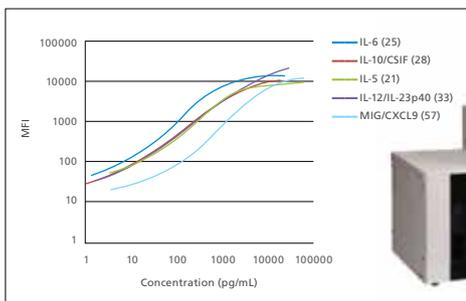


Label Detection

- For analyte quantitation, the immuno (sandwich) complex is incubated with a fluorescent detection label (SA-PE).

Incubate 30 min and wash

Figure 4



Read & Analyze

- Read with Luminex 100/200 or Magpix instrument.
- Analyze results.

Secreted proteins simultaneously measured using Procarta Cytokine Assay.

Contract Manufacturing

eBioscience offers commercial contract manufacturing services for large-scale antibody and protein production in our certified facility, to cGMP, ASR, or ISO CE-IVD regulatory requirements. You can provide us with one of your clones or customize one of ours.

- Customer defines production specifications
- Select conjugations to biotin, HRP or more than 20 different fluorochromes

Custom ASR Antibody Production*

eBioscience is registered with the U.S. Food and Drug Administration as a Medical Device Class 1 manufacturer for Analyte Specific Reagent (ASR) products.

ASR products include antibodies and proteins for

- *In vitro* diagnostic manufacturers
- Clinical laboratories

eBioscience custom-manufactures ASR antibodies that fully comply with Federal regulations 21CFR820 and 21CFR864.4020. According to 21CFR809.30(d), Class I ASRs must be labeled as “Analyte Specific Reagent. Analytical and performance characteristics are not established.”

CE-IVD and ASR Build-on-Demand*

Our “Build-on-Demand” program allows for an accelerated custom service at off-the-shelf pricing. eBioscience has the ability to provide ASR- or CE-labeled product within 3-4 months under the following conditions:

- IVD rights are secured for the desired clone
- Build-on-Demand formats are selected

Build-on-Demand Formats				
Laser	Build-on-Demand Formats	Emission	Equivalent Dye	Emission
Violet (405 nm)	eFluor® 450	450 nm	Horizon™ V450 Pacific Blue®	448 nm, 452 nm
Blue (488 nm)	FITC	518 nm	Alexa Fluor®488	519 nm
	PE	575 nm	RD1	575 nm
	PerCP	675 nm	PE-Cy5	670 nm
	PerCP-Cy5.5	690 nm	PE-Cy5.5	667 nm
	PerCP-eFluor® 710	710 nm	PerCP-Cy5.5, PE-Cy5.5	690 nm, 667 nm
	PE-Cy7	785 nm	PC7	785 nm
Red (633 nm)	APC	660 nm	-	-
	APC-eFluor® 780	780 nm	APC-H7, APC-Alexa Fluor®750	785 nm, 780 nm

*Not all products available in all regions. Please contact customer service for more information.

Quality First. Value Always.

eBioscience understands your need for antibodies to perform optimally every time. Reagents that consistently provide optimal signal, with low background and reproducible lot-to-lot performance save you both time and money while eliminating the need to repeat testing due to reagent failure. eBioscience ensures the integrity of your long-term studies. Our antibodies for flow cytometry are developed using well-defined performance criteria in relevant model systems and are rigorously tested to provide consistent quality, performance and value.

Our scientists are committed to keeping pace with the dynamic changes in the life sciences by developing relevant, high-quality reagents at an accelerated pace. We pride ourselves on being consistently first-to-market with many critical reagents. eBioscience reagents are packaged in convenient sizes to accommodate every study design, and are supported by a highly respected technical support group staffed by experienced scientists to ensure that eBioscience customers receive the maximum return on their reagent investment.

With eBioscience, feel confident you are getting both the best product and overall value to support your studies.

Industry Leading Performance

As part of our commitment to providing you with optimal performing reagents, we routinely evaluate eBioscience antibody performance against competitive products when the same clone and format are commercially available. We strive to offer reagents that perform better than comparable products available from other suppliers.

Lot-to-Lot Consistency

In addition to optimal performance, reagents from eBioscience demonstrate dependable lot-to-lot consistency. This commitment to quality ensures reliable data for long-term studies, and eliminates the need to recalibrate your staining for a new lot of antibody. Our stringent manufacturing specifications require consistent performance between production lots to ensure reliable and accurate results in your studies.

How to order

1

Contact your Regional Account Manager, or visit our website to submit a request

2

Discuss your project with our in-house consultation team

3

eBioscience provides the answer to your research needs with a custom solution

www.eBioscience.com/custom

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