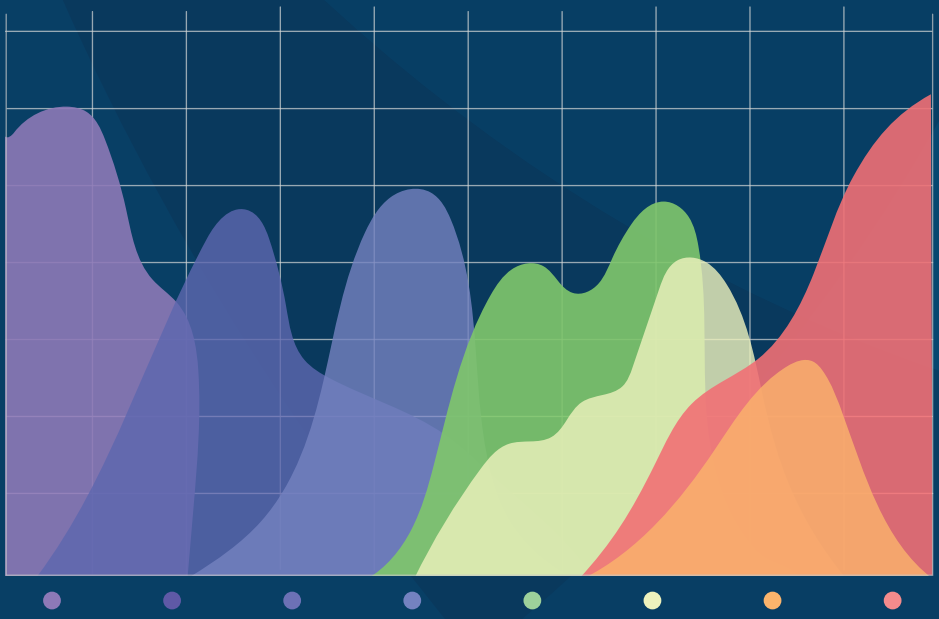
proteintech®
Antibodies | ELISA kits | Proteins

The Complete Guide To

FLOW CYTOMETRY



www.ptglab.com

CONTENTS

5 Introduction

6 Fluorophores and dyes

9 Flow cytometry multicolor panel design

10 Compensation control in flow cytometry

11 Controls in flow cytometry

12 Sample preparation

15 Protocol for studying extracellular and intracellular proteins

17 Protocol for studying cell viability and apoptosis

19 Troubleshooting

20 Antibodies for flow cytometry

22 Further resources and support

24 Contact us

WELCOME

This booklet aims to give you a complete guide to flow cytometry. It will cover a comprehensive background to flow cytometry, step-by-step protocols and useful technical tips, and troubleshooting.

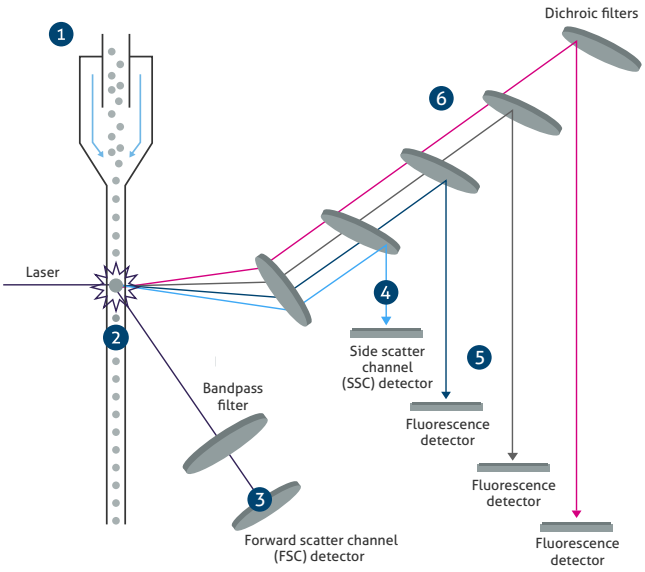
Flow cytometry is a technique that enables the measurement of properties of individual particles (cells) in a heterologous population. This allows researchers to phenotype each cell based on these properties and in some cases also to sort cells into different subpopulations for further evaluation in specialized flow cytometry called fluorescence-activated cell sorting (FACS). In a flow cytometer, cells in suspension are forced into 'single file' to then pass through a laser, which enables the measurement of particle size and granularity. Further phenotyping occurs through using fluorescent conjugated antibodies that bind to target proteins on the cell surface or intracellularly, enabling the measurement of fluorescence intensity as another parameter.

Flow cytometry applications

- **Analysis of plasma membrane receptor expression**
- **Analysis of cell viability, apoptosis, and necrosis**
- **Cell phenotyping**
- **Analysis of cell cycle**
- **Cell sorting**
- **Analysis of intracellular protein expression**
- **Biomarker detection**
- **Cell counting**

HOW DOES FLOW CYTOMETRY WORK?

Figure 1 **Overview of a flow cytometer**



Introduction

In general, a flow cytometer is composed of three parts: the fluidics system, optics, and electronics. The fluidics system enables hydrodynamic focusing of a single stream of particles by forcing the cell suspension through the sheath towards a narrow nozzle, generated by the force of the enclosed outer sheath fluid, Figure 1 (1). This hydrodynamic focusing enables cells to pass one-by-one through one or more light beams (2). Light scattered in the forward direction to that of the laser is then collected by a photomultiplier tube (PMT) and is referred to as the forward scatter channel (FSC) (3). This measurement provides information estimating the size of the particle as in general, larger particles refract more light than smaller ones. PMTs measuring light at roughly 90° to the light beam are called the side scatter channel (SSC) (4). This provides information on the relative granularity of the cell. For example, neutrophils are highly granular cells in comparison to macrophages.

Fluorescent light detected at different wavelengths by PMTs (5) provides further information for cell identification based on the combination of fluorescently tagged cell markers. This is achieved by the use of specialized filters that can transmit certain wavelengths of light while blocking other wavelengths. There are three main types of filters: short pass filters that allow wavelengths of light below a specified threshold, long pass filters that allow wavelengths of light above a specified threshold, and dichroic mirrors (6) that allow certain wavelengths to pass through but reflect others. Therefore, using a combination of these filters in a certain order enables the detection of multiple signals simultaneously.

When a particle passes through a laser, it creates a pulse of information, which is termed an 'event.' Each event can be analyzed for its size (area under the curve; the time it takes for the particle to pass through the beam) and signal intensity (height). Thresholds for these parameters are set in order to determine which events correspond to cells, rather than smaller particles, which will be ignored by the detectors. Fluorescence data is displayed in a logarithmic scale to facilitate easy visualization on a histogram due to the wide range in signal distribution by strong and weak signals. Each parameter is displayed according to its height, area, and width by specialist software. This is then interpreted to compare populations according to your experimental design.

Fluorophores and dyes

The majority of flow cytometry experiments involve the use of fluorophores. They act by accepting energy of a certain wavelength (for example a laser) that causes the electrons in the fluorophore to move from a resting to an excited state, releasing energy as fluorescence at a lower wavelength. The excitation-emission spectrum of a fluorophore defines its properties when used for designing panels in multiplex experiments. When conjugated to primary or secondary antibodies, fluorophores in flow cytometry can be used to identify and quantify populations of cells based on targeted cell surface markers or intracellular targets. Cells manipulated to express fluorescent proteins such as GFP or Venus can use their fluorescent properties in a similar manner, with the added benefit that these can be used to tag intracellular markers in live cells without the need for membrane permeabilization.

Single dye organic fluorophores such as FITC, APC, PE, and PerCP have been around for many years. More recently, synthetic dyes such as Alexa Fluor™1 and CoraLite have been designed with enhanced brightness and greater photostability. Tandem dyes are also available and commonly used in flow cytometry, whereby two fluorophores are covalently coupled together. When the first dye (donor) is excited, it transfers energy to the second dye (acceptor), which then becomes activated, resulting in fluorescence. Thus, tandem dyes enable more colors to be detected from a single laser wavelength.

Table 1 Excitation-emission details of common fluorophores and their relative brightness

Fluorophore	Fluorescence Colour	Maximal Absorbance, nm	Maximal Emissions, nm	Relative Brightness
Alexa Fluor®405		401	421	3
Pacific Blue		410	455	1
CoraLite®488		495	519	3
FITC		490	525	3
PE*		490; 565	578	5
CoraLite® 594		590	617	4
APC		650	661	4
CoraLite®647		650	665	4
PerCP		490	675	2
Alexa Fluor®700		702	723	2

*PE is the same as R-phycoerythrin

Cy = cyanine. APC = allophycocyanin. FITC = fluorescein isothiocyanate. PE = phycoerythrin. PerCP = peridinin chlorophyll protein.

Choosing a fluorophore

There are many different types of fluorophores available, and their different properties can be used to best suit the desired targets and the experimental setup:

1. **To minimize spectral overlap, avoid using fluorochromes with similar excitation spectra if they are excited by the same laser.**

2. **Use bright fluorophores with low expression markers to resolve their signal from background noise, whereas abundantly expressed markers can use dyes that are less bright.**

3. **Some fluorochromes add significant molecular weight to the labeling reagents – this may reduce their ability to permeate the cell membrane to stain intracellular targets.**

4. **Be sure to properly handle tandem dyes – avoid freeze-thaw cycles and exposure to light to minimize decoupling.**

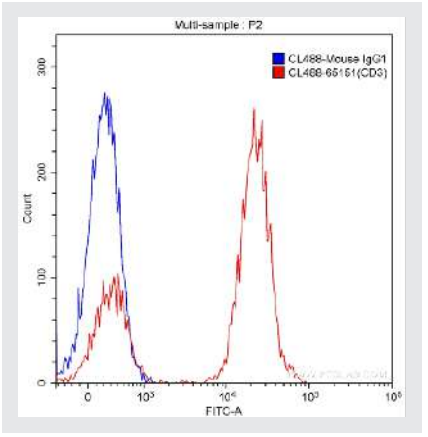
If fluorophore reagents are used, cells are stained and then washed prior to analysis to remove the excess of unbound dyes. Please see Table 2 for tips on staining procedures with different fluorophore reagents. Permeabilization is required for the analysis of intracellular proteins using antibodies.

1 Alexa Fluor® is a registered trademark of Thermo Fischer Scientific.

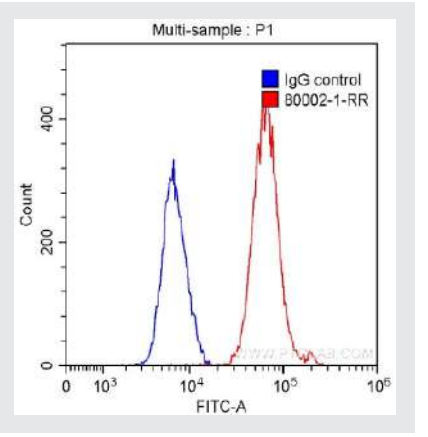
Table 2 Types of fluorophore reagents commonly used in flow cytometry

Reagent	Example	Live cells	Fixed cells	Permeabilization
Viability dyes	Propidium iodide	✓	✗	✗
Apoptotic markers	Fluorescently labeled annexin V	✓	✗	✗
Fluorescent proteins expressed by cells	GFP-LC3	✓	✓	Not required
Fluorescently labeled antibodies against extracellular proteins	CD3 (see Figure 5)	✓	✓	Not required
Fluorescently labeled antibodies against intracellular proteins	TDP-43 (see Figure 5)	Technically challenging	✓	Required

Figure 2 Flow cytometry is suitable for analyzing both extracellular and intracellular targets



Left: 1X10^6 human peripheral blood lymphocytes were surface-stained with 5.00 ul/ test CoraLite®488-conjugated Anti-Human CD3 (CL488-65134, Clone: OKT4) (red) or control antibody (blue). Cells were not fixed.



Right: 1X10^6 HeLa cells were intracellularly stained with 0.20ug Anti-Human TDP-43 (80002-1-RR, Clone:16A22) (red) or 0.20 ug control antibody (blue) and CoraLite®488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) with dilution 1:1000. Fixed with 90% MeOH.

Flow cytometry multicolor panel design

Many flow cytometry experiments use more than one fluorophore to stain samples. Multicolor experiments are a powerful tool that not only enable cost savings by analyzing fewer samples at a time but also allow for the gathering of more information using single cell analysis. They also make it possible to examine relationships between targets – e.g., examining whether cells positive for one target also express the other one. However, multicolor experiments require careful design.

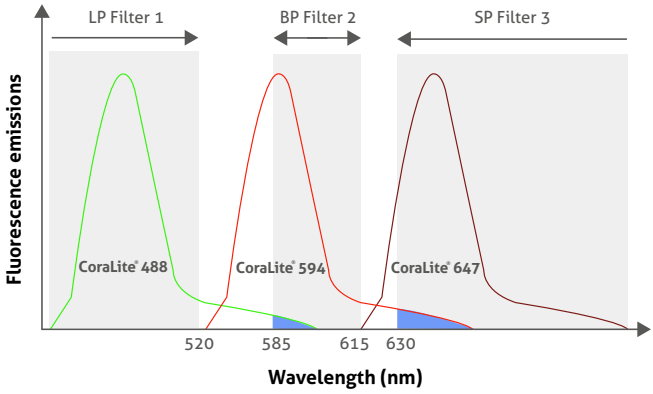
Filters in flow cytometers

Every fluorophore has characteristic excitation and emission spectra. The wavelength of the laser light is chosen based on its excitation spectrum. Prior to detection, the emitted light is filtered through sets of optical filters. There are three types of filters:

- **longpass (LP) – filter light above a certain wavelength**
- **bandpass (BP) – filter light between certain wavelengths**
- **shortpass (SP) – filter light below a certain wavelength**

It is easy to choose appropriate filters in single fluorophore experiments. However, in multicolor experiments with fluorophores excited by the same laser, sets of filters need to be chosen with caution. The example setup depicted in Figure 3 shows the emission spectra of three fluorophores. SP filter 1 filters light below 520 nm, which allows it to gather the spectrum of fluorophore 1. BP filter 2 (600/15 nm) collects light from fluorophore 2, while LP filter 3 (630 nm) collects most of the spectrum of fluorophore 3. Using appropriate filter sets, the user is able to efficiently collect signals originating from different fluorophores.

Figure 3 **Emission spectra of CoraLite® fluorophores – considerations for light filtering and compensation using LP, BP, and SP filters**



Compensation controls in flow cytometry

The emission spectra of different fluorophores often overlap (Figure 3 – marked in blue), which means that additional fluorescence compensation is needed. In the example given, filters 2 and 3 detect some of the light emitted from fluorophores 1 and 2 respectively. Therefore, in order to ensure which cells in the analysis are truly positive or negative for a given fluorophore, compensation is required. There are two types of compensation controls: 1) single stained controls and 2) fluorescence minus one (FMO) controls. Compensation can be computationally applied after running all samples based on the results from performing these two controls.

Single stained controls are cell samples or beads that are stained with only one fluorophore. Cells are good for compensation provided that they are a mix of both positive and negative cells for a certain fluorophore reagent. This way it is possible to distinguish between positive and negative events for a single channel. Commercially available compensation beads are an alternative to single stained cell controls. They are composed of a mix of beads, where a proportion of them have binding sites for antibodies. They often provide a better distinction of negative and positive events and permit the more efficient usage of scarce cell sample to set up controls, but are not compatible with all antibody species. Single stained samples should be performed for every fluorophore used.

Voltage settings are important for the correct interpretation of results. Voltages are set so that FSC and SSC settings are able to exclude cell debris. Actual settings depend on the experimental setup and should first be adjusted with single stained control samples to make sure that all events are within the scale.

FMO controls are vital in experiments using more than two fluorophores. FMO controls are samples stained with all used fluorophores in the experiment apart from one. They are important for assessing fluorescence spreading and for final gating.

Table 3 Example experimental design for FMO controls

ANTIGEN	CoraLite® 488	PE	Cy5-PE	APC
Unstained	-	-	-	-
CD3 FMO	-	CD4	CD8	CD19
CD4 FMO	CD3	-	CD8	CD19
CD8 FMO	CD3	CD4	-	CD19
CD19 FMO	CD3	CD4	CD8	-

***Table 3** demonstrates a typical experimental design for unstained and FMO controls in a multiplex panel. The '-' indicates which antibody(s) is removed from each control.*

Controls in flow cytometry

Irrespective of whether the experiment is multicolor or not, there are additional controls that should be included in all flow cytometry experiments.

Unstained controls are required in order to assess the level of cell autofluorescence in your sample, to which you can then appropriately set your voltage and negative gates to account for this.

Isotype controls utilize antibodies raised against antigens that are not present in cells, and are used as a form of negative control, in place of the target antibody, to assess the level of non-specific binding. These controls should be used with caution, and must be the same host, class, and subclass, conjugated to the same fluorophore and used at the same working concentration. If all other controls are performed, then an isotype control may not be necessary.

Fc block controls are performed using species-specific serum, such as mouse or human IgG or commercial reagents. This prevents Fc mediated binding of conjugated target antibodies to Fc receptors on certain cell types such as B lymphocytes, NK cells, granulocytes, monocytes, macrophages, and platelets. This non-specific Fc binding can cause false positive results; the Fc block thus ensures that the observed signal is specific to antibody-paratope/cell-epitope binding.

Biological controls should include negative and positive samples for your target antigen known in the literature, or cell lines where the target has been overexpressed or knocked down (e.g., siRNA constructs) or out (e.g., CRISPR constructs). These controls are necessary to ensure that your protocol is working correctly and importantly validates any negative results. In experiments where stimulation is required in order to observe the target antigen, a non-stimulated sample must also be included.

The necessary controls for flow cytometry are summarized in Table 4 - Opposite page.

Table 4 Controls in flow cytometry experiments

Control	Reason to include
Single stained control	To account for spectral overlap
Fluorescence minus one (FMO) control	To adjust for spectral overlap in multicolor experiments
Unstained control	To account for cell autofluorescence
Isotype control	To account for non-specific binding with a species-matched antibody
Fc block control	To block Fc receptors in immune cells – essential in staining B cells, dendritic cells, monocytes, and macrophages
Biological control	To ensure that the staining procedure is successful

Sample preparation

As in any antibody-based technique, biological samples require preparation prior to staining and analysis. Cells require disassociation and are often fixed and permeabilized when analyzing cytoplasmic and nuclear proteins. Samples need to be washed after staining to remove any excess of unbound fluorescent reagents. Please refer to our experimental workflow (Figure 4) and sample preparation table (Table 5).

Figure 4 Experimental workflow for analyzing protein targets in flow cytometry

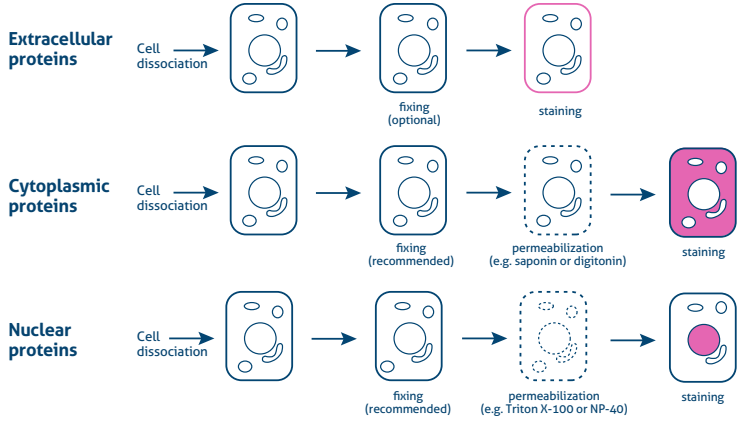


Table 5 Sample preparation steps in flow cytometry experiments

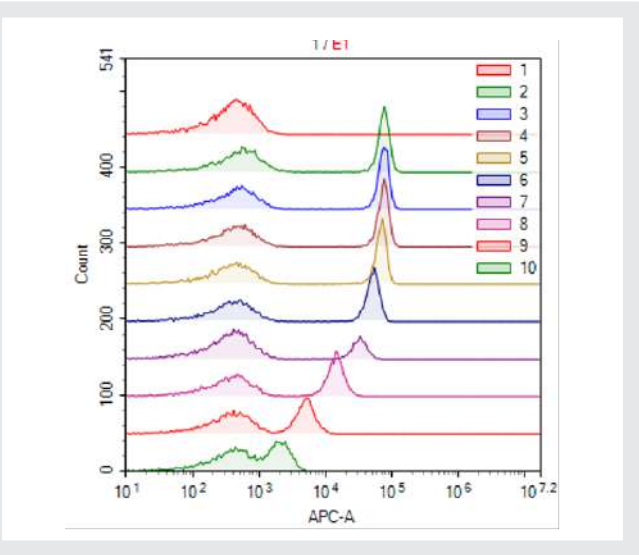
Sample preparation step	Description and tips
Cell dissociation	• Adherent cells need to be detached prior to flow cytometry analysis using detaching agents, e.g., trypsin. Milder enzymes are frequently used for cell detachment in order to preserve plasma membrane receptors if they are a subject of analysis. • Use appropriate buffers to prevent cell clumping – avoid calcium and magnesium salts. If they are present, a chelating agent, e.g., EDTA, can be added to samples. • Tissue samples require more stringent dissociation methods – either mechanical or enzymatic (e.g., trypsin, collagenase).
Fixation	• Advantageous for sample preservation but can increase autofluorescence levels. • Two types of fixatives available: alcohols (methanol or ethanol) and aldehydes (formaldehyde or glutaraldehyde).
Permeabilization	• Necessary for staining of intracellular proteins. • Mild detergents, such as saponin or digitonin, are used for analysis of cytoplasmic proteins. • Harsher detergents, e.g., Triton X-100 or NP-40, need to be used for analysis of nuclear proteins to ensure permeability of both the plasma membrane and the nuclear envelope.
Incubation with fluorophores, dyes, or antibodies	• Optimization of used concentrations needed to achieve best signal to noise ratio and to reduce non-specific binding. • Choice of dyes influences other steps (e.g., compatibility with used fixatives). • Careful design needed for multicolor analyses (see section 4).
Washing	• It is necessary to remove remnants of fixatives, permeabilization agents, and unbound fluorophores.

The proper execution of sample preparation steps helps to reduce noise and ensure good-quality data are obtained. The presence of many dead cells, cell clumps, and high cell autofluorescence can usually be avoided. For example, the presence of dead cells can be spotted by analyzing FSC vs SSC plots – dead cells have low FSC and high SSC compared to live cells. It is good practice to include viability dyes in experiments as this provides a definite method of excluding dead cells from analysis. Dead cells are particularly sticky to antibodies and can therefore significantly influence and falsify results if they are not excluded.

Optimal antibody concentration

It is also important to optimize the dilutions of antibodies and other probes used for staining. This not only allows the saving of reagents but also reduces the background from non-specific binding. The correct dilution depends on the antibody affinity to the target and target abundance. Very high dilutions are recommended for abundant targets. As seen in Figure 5, it is possible to easily discriminate between CD4-positive and CD4-negative lymphocytes stained with even very high dilutions of the anti-CD4 antibody.

Figure 5 Dilution of antibodies for sample staining in flow cytometry



1X10^6 human peripheral blood lymphocytes were surface-stained with (1) APC-Mouse IgG2b isotype control at 0.25 µg, or (2-10) APC-Anti-Human CD4 (APC-65134, clone OKT4) using 2-fold serial dilutions from 0.25 µg to 10-4 µg. Samples were not fixed.

Protocol for studying extracellular and intracellular proteins

Materials and equipment:

- Cell dissociation agent (e.g., trypsin) – for adherent cell lines only
- Cell culture medium containing FBS or trypsin inhibitors
- PBS or HBSS
- Benchtop centrifuge
- Fixative (e.g., 3% (w/v) Paraformaldehyde (PFA))
- Permeabilization solution (e.g., 0.1% (w/v) saponin or 0.1% (w/v) Triton X-100)
- Primary and secondary antibodies
- Flow cytometer

Experimental procedure:

1. Adherent cells:
   a. Remove medium and wash cell monolayer with PBS.
   b. Add dissociating agent (e.g., trypsin) and incubate at 37°C for 5 min or until cells detach from the cell culture dish.
   c. Add serum-containing medium or trypsin inhibitors to inactivate dissociating agent.
   d. Transfer cells into a microcentrifuge tube.
   Suspension cells: Transfer cells into a microcentrifuge tube.

2. Pellet cells by centrifugation (300 G's, 5 min at room temperature). Remove medium and resuspend in PBS or HBSS.

3. Count cells and take 1 million (10^6) cells per condition:
   a. Unstained sample – for establishing autofluorescence levels
   b. Sample stained with antibody A
   c. Sample stained with antibody B
   d. Sample stained with both antibodies

Note 1: Consider an isotype control to compensate for non-specific binding with a species-matched antibody.

Note 2: Fluorophores conjugated to antibodies A and B have to have different excitation spectra in order to distinguish their staining. Please refer to the section on multicolor design experiments.

4. Optional: fix cells with a fixative agent (e.g., incubate with 3% (w/v) paraformaldehyde (PFA) solution for 20 min at room temperature). Centrifuge as in step 3 and wash cells with PBS.

Note: This step is recommended for staining intracellular targets.

5. For intracellular targets, permeabilize cells.
   a. For cytosolic protein staining, use milder detergents – e.g., incubate with 0.1% (w/v) saponin solution for 5-10 min.
   b. For nuclear protein staining and proteins inside cellular organelles, use harsher detergents – e.g., incubate with 0.1% (w/v) Triton X-100 solution for 5 min. Centrifuge as in point 3 and wash cells with PBS or HBSS.

6. Add the antibodies A and/or B according to requirements in step 3, and incubate for 20 min at room temperature (include milder detergent if it was used for permeabilization).

7. Pellet cells by centrifugation (300 G's, 5 min at room temperature). Wash cells twice with PBS or HBSS.

8. If using unlabeled primary antibodies, incubate with secondary antibodies for 20 min at room temperature. Wash cells as in noted in step 7.

Note: When using secondary antibodies, primary antibodies have to be raised in different species to distinguish their staining. This is not a prerequisite when using fluorophore-conjugated primary antibodies because no secondary antibodies are needed for detection.

9. Analyze cells on a flow cytometer.

Protocol for studying cell viability and apoptosis

Materials and equipment:

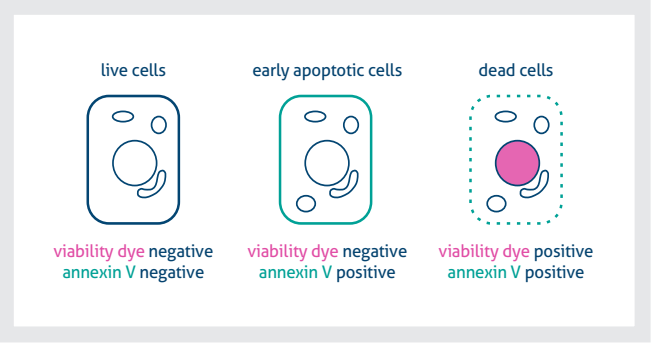
- Cell dissociation agent (e.g., trypsin) – for adherent cell lines only
- Cell culture medium containing FBS or trypsin inhibitors
- PBS or HBSS supplemented with calcium chloride
- Benchtop centrifuge
- Fluorescent annexin V probe
- Viability dye (e.g., propidium iodide)
- Flow cytometer

Experimental procedure:

Cell viability experiments using flow cytometry are performed quite commonly. They are used to study the effect of different agents, e.g., cytotoxic drugs, on the cell viability of various cell lines. They can be used to establish the minimum required concentration of antibiotics causing cytotoxic effects, which is useful for stable cell generation using antibiotic markers. They are often used when studying apoptosis and other forms of cell death. Viability dyes are recommended in flow cytometry experiments and are performed on unfixed cells in order to exclude dead cells from the analysis.

Live cells and early apoptotic cells are impermeable to viability dyes because they have an intact cell membrane and therefore are negative (Figure 6). Annexin V binds to phosphatidylserine that is found on the outer layer of early apoptotic cells. Annexin V also binds to dead cells, where it binds to a pool of phosphatidylserine present on the outer and inner layers because of their compromised cell membrane integrity. Viability dyes are able to penetrate dead cells and bind to their DNA.

Figure 6 Analysis of cell viability and apoptosis by flow cytometry



Cell viability and apoptosis protocol

1 Adherent cells:

a. Remove medium and wash cell monolayer with PBS.

b. Add dissociating agent (e.g., trypsin) and incubate at 37°C for 5 min or until cells detach from the cell culture dish.

c. Add serum-containing medium or trypsin inhibitors to inactivate dissociating agent.

d. Transfer cells into a microcentrifuge tube.
Suspension cells: Transfer cells into a microcentrifuge tube.

2. Pellet cells by centrifugation (300 G's, 5 min at room temperature). Remove medium and resuspend in PBS or HBSS containing calcium ions.

Note: Annexin V requires calcium for interaction with phospholipids. Supplement buffers with calcium salts and avoid chelating agents such as EDTA or EGTA.

3. Count cells and take 1 million (10^6) cells per condition:

a. Unstained sample – for establishing autofluorescence levels.

b. Sample stained with a viability dye.

c. Sample stained with an annexin V probe.

d. Sample stained with a viability dye and an annexin V probe.

Note 1: Annexin V and a viability dye have to have different excitation spectra in order to distinguish their staining. Please refer to section 4 for tips on multicolor design experiments.

Note 2: Consider including a positive control sample, where cells are treated with an agent inducing cell death to validate the performance of used probes.

4. Add fluorescently labeled annexin V and incubate for 15 min at room temperature. Annexin V preferentially binds to phosphatidylserine, which is found on the inner layer of the plasma membrane in living cells. In early apoptotic cells, phosphatidylserine is translocated to the outer layer, making it accessible for annexin V binding.

5. Pellet cells by centrifugation (300 G's, 5 min at room temperature). Remove medium and resuspend in PBS or HBSS.

6. Add a viability dye (e.g., propidium iodide) and incubate for 5-20 min at room temperature. Propidium iodide (PI) is a DNA intercalating agent – it is used as a fluorescent dye that binds to DNA. Dead cells lose cell membrane integrity, which allows the dye to reach the nucleus and bind to nucleic acids.

7. Analyze cells on a flow cytometer.

Note: Do not wash cells prior to analysis to avoid washing out the viability dye accumulated in dead cells.

Troubleshooting

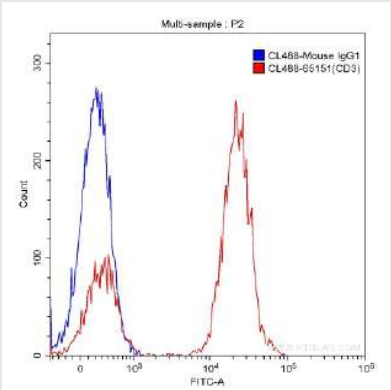
Problem	Possible cause and solutions
No or weak signal	• **Incorrect antibody storage** Ensure that all antibodies are stored according to the manufacturer's instructions. E.g., PE should not be frozen. • **Wrong dilution** Perform a titration to check required antibody concentration. • **Incorrect laser** Confirm correct laser is in use and check laser alignment. • **Intracellular target is inaccessible or fluorochrome is too large** Ensure adequate permeabilization and change fluorochrome to a low MW. • **Target protein has low or no expression** Use a positive control to check staining procedure and use a brighter fluorophore for the target. • **Soluble target protein has been excreted** Implement a Golgi-block step.
Non-specific staining	• **Autofluorescence** Include an unstained sample control. • **Fc mediated binding** Include an Fc block control for B cells, dendritic cells, monocytes, and macrophages. • **Insufficient washing** Add more wash steps. • **Too low antibody concentrations** Test-titrate antibodies, non-specific binding favored at lower concentrations.
High fluorescence intensity	**Antibody concentration too high** Perform a titration to check required antibody concentration. **Insufficient blocking** Increase blocking reagent and include blocking reagent with antibody incubation buffer. **Antibody trapped for intracellular targets** Increase number of wash steps and add low concentrations of a permeabilization reagent in wash buffers.
Non-specific staining	**Lysed or broken cells** Use fresh samples, avoid excessing vortexing and high centrifuge speeds. **Activation** Some activation methods affect scatter profiles.

Antibodies for flow cytometry

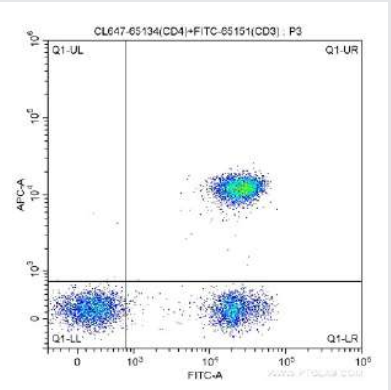
CoraLite® 488-Anti-Human CD3 (CL488-65134, clone OKT4)

CD3 is a complex of proteins (gamma, delta, and two epsilon chains) that directly associates with the T-cell receptor (TCR) to regulate T-cell activation. The TCR-CD3 complex is responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. Once TCR-mediated peptide-MHC binding occurs, the signal is transmitted to the CD3 complex, leading to intracellular signal transduction. CD3 is a pan-T cell marker for the detection of normal and neoplastic T cells.

Figure 7 CoraLite® 488-Anti-Human CD3 (CL488-65134, clone OKT4) in flow cytometry

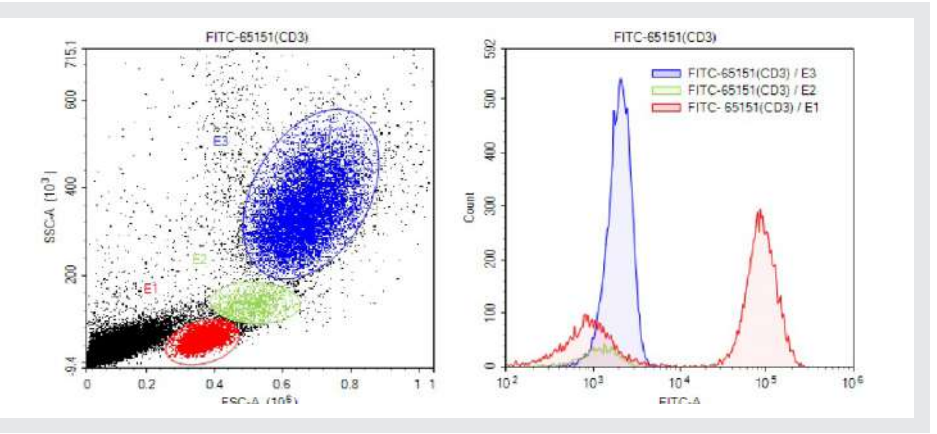


Left: 1X10^6 human peripheral blood lymphocytes were surface-stained with 5.00 ul/test CoraLite®488-conjugated Anti-Human CD3 (CL488-65134, Clone: OKT4) (red) or control antibody (blue).



Right: 1X10^6 human peripheral blood lymphocytes were surface-stained with FITC Anti-Human CD3 (FITC-65151, Clone: UCHT1) and 5.00 ul CoraLite®647-conjugated Anti-Human CD4 (CL647-65134, Clone: OKT4).

Figure 8 FITC-Anti-Human CD3 (FITC-65151, clone UCHT1) in flow cytometry

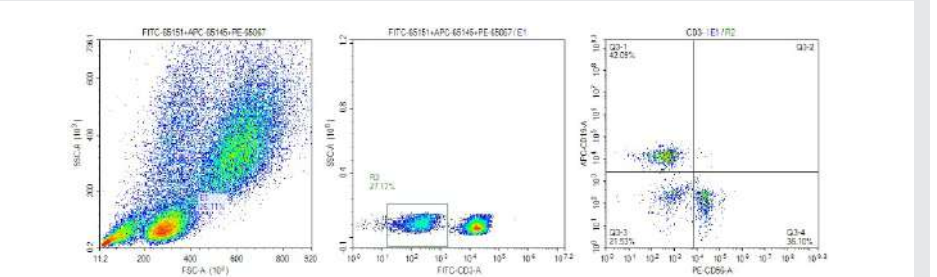


0.1 mL human whole blood cells were surface-stained with FITC-Anti-Human CD3 (FITC-65151, clone UCHT1). Samples were then treated with red blood cell lysis buffer and were gated for analysis of CD3 staining. Cells in the lymphocyte (red histogram), monocyte (green histogram), or granulocyte (blue histogram) gates (E1, E2, E3) were used to compare CD3 staining.

APC-anti-human CD19 (APC-65145, clone SJ25C1)

CD19 is a B cell-specific molecule that controls B cell activation by complexing with the B cell receptor (BCR). CD19 is a member of the Ig superfamily and is the dominant component for the signaling complex on B cells that includes CD21, CD81, and CD22. CD19 deficiency leads to reduced proliferative responses to BCR stimulation in vitro and reduced antibody production following vaccination.

Figure 9 APC-anti-human CD19 (APC-65145, clone SJ25C1) in flow cytometry

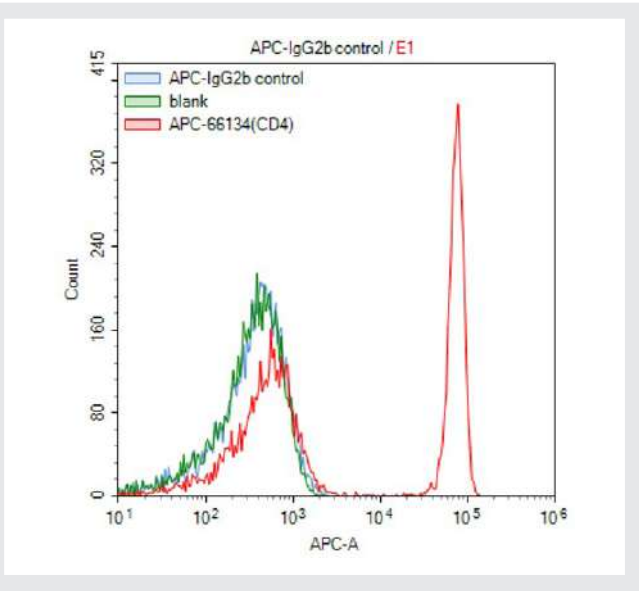


0.1 mL human whole blood cells were surface-stained with 0.20 ug FITC-Anti-Human CD3 (FITC-65151, clone UCHT1), 0.20 ug APC-anti-human CD19 (APC-65145, clone SJ25C1), and PE-Anti-Human CD56 (PE-65067, clone MEM 188). Samples were then treated with red blood cell lysis buffer and were gated for CD3 negative lymphocytes for analysis of CD19 and CD56 staining.

APC-Anti-Human CD4 (APC-65134, clone OKT4)

CD4 is a 55-kDa transmembrane glycoprotein expressed on T helper cells, the majority of thymocytes, monocytes, macrophages, and dendritic cells. CD4 is an accessory protein for major histocompatibility complex (MHC) class-II antigen/T-cell receptor interaction. It plays an important role in T helper cell development and activation. CD4 serves as a receptor for human immunodeficiency virus (HIV).

Figure 10 APC-Anti-Human CD4 (APC-65134, clone OKT4) in flow cytometry



1X10^6 human peripheral blood lymphocytes were surface-stained with 0.20 ug APC-Anti-Human CD4 (APC-65134, clone OKT4) (red) or 0.20 ug APC-Mouse IgG2b isotype control (blue) and blank control (green). Samples were not fixed.

Further resources and support

If you have any questions regarding these protocols or any of our products for flow cytometry, our expert technical support team are always happy to help. Contact **proteintech@ptglab.com** or visit our website **www.ptglab.com** for more contact details.

For further detailed information on flow cytometry principles and protocol, please see this highly comprehensive review by Cossarizza et al. 2019, Eur J Immunol (PMID: 31633216). Additionally, for more specialized protocols we recommend: Chow et al. 2003, Cytometry Part A (PMID: 16080188) for a whole blood fixation and permeabilization protocol, and Krutzik and Nolan, 2003, Cytometry Part A (PMID: 14505311) for post-translational modifications such as phosphorylation.

proteintech®

Antibodies | ELISA kits | Proteins

FROM OUR BENCH TO YOUR BENCH™

Discover the benefits of antibodies from a manufacturer-direct company for reproducible, reliable data:

- **Extensively validated in WB, IHC, IF, IP & more.**
- **Raised against whole protein immunogens.**
- **Guarantee covers any species/application.**
- **Antibody specificity demonstrated by siRNA knockdown.**

Proteintech® has over 13,000 antibodies in its extensive catalog, all fully validated and available for next day delivery.

www.ptglab.com

CONTACT US

Proteintech Group
US Head Office

Phone
1 (888) 4PTGLAB
(1-888-478-4522)
(toll free in USA),
or 1(312) 455-8498 (outside USA)

Email
proteintech@ptglab.com

Proteintech Europe
United Kingdom

Phone
0161 839 3007

Email
europe@ptglab.com

Proteintech Europe
Germany

Email
germany@ptglab.com

Sales and technical support only.

Proteintech
China Office

Phone
027-87531629
027-87531627
or 4006-900-926

Email
Proteintech-CN@ptglab.com

proteintech®
Antibodies | ELISA kits | Proteins

We are ISO 9001 and
ISO 13485 accredited.

Support

Available 24 hours a day
via email or 9-5 via phone.

You can also speak to
a representative at any
time via Live chat:
www.ptglab.com.

Live Chat
www.ptglab.com

Twitter
@proteintech

Blog
ptglab.com/news/blog

YouTube
www.youtube.com/Proteintech