Kit Manual

BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit

(Cat. No. 554714)

BD Cytofix/Cytoperm™ Plus Fixation/Permeabilization Kit (with BD GolgiStop™ protein transport inhibitor containing monensin)

(Cat. No. 554715)

BD Cytofix/Cytoperm™ Plus Fixation/Permeabilization Kit (with BD GolgiPlug™ protein transport inhibitor containing brefeldin A)

(Cat. No. 555028)

BD

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BD Biosciences offers three kits to simplify the fixation and permeabilization of cells for immunofluorescent staining of intracytoplasmic cytokines. The **BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit** provides a fixation/permeabilization solution and a permeabilization/washing solution. The **BD Cytofix/Cytoperm™ Plus Fixation/Permeabilization Kit (with BD GolgiStop™ protein transport inhibitor)** provides these two solutions plus a protein transport inhibitor containing monensin for the treatment of freshly-explanted or cultured cells to promote intracytoplasmic cytokine accumulation. The **BD Cytofix/Cytoperm™ Plus Fixation/Permeabilization Kit (with BD GolgiPlug™ protein transport inhibitor)** provides the two solutions, plus an alternative protein transport inhibitor containing brefeldin A. These kits provide enough of the two solutions for staining ≥ 200 cell samples.

BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit (Cat. No. 554714)

This kit enables the fixation and permeabilization of cells which is necessary for staining intracellular cytokines with fluorochrome-conjugated anti-cytokine antibodies. The kit provides two reagents, fixation/permeabilization solution and BD Perm/Wash™ Buffer. After cell fixation and permeabilization, the BD Perm/Wash™ Buffer is used to wash the cells and to dilute the anti-cytokine antibodies for staining.

Note: It is important that the BD Perm/Wash™ Buffer be used for dilution of anti-cytokine antibodies, rather than a standard staining buffer, in order to maintain cells in a permeabilized state for intracellular staining.

Kit components:

- Fixation/Permeabilization solution (125 ml)
- BD Perm/Wash™ Buffer, 10 × concentrate containing Fetal Bovine Serum (FBS)* and saponin (dilute 1:10 in distilled H_20 prior to use) (100 ml)

Note 1: Although both the Fixation/Permeabilization solution and the BD Perm/Wash™ Buffer contain constituents that prevent contamination, it is recommended that the solutions be removed using sterile pipettes and the bottles closed immediately after use.

Note 2: BD Perm/Wash™ buffer is supplied as 10× stock solution that contains FBS*. The color of this product may vary from lot-to-lot, and it may contain visible precipitate. Color variation and/or precipitate do not affect product performance.

** Source of all serum proteins is from USDA inspected abattoirs located in the United States.*

BD Cytofix/Cytoperm™ Plus Fixation/Permeabilization Kit (with BD GolgiStop™ protein transport inhibitor) (Cat. No. 554715)

In addition to the Fixation/Permeabilization solution and BD Perm/Wash™ Buffer included in the BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit, the BD Cytofix/Cytoperm Plus Fixation/Permeabilization Kit provides BD GolgiStop protein transport inhibitor containing monensin. The *ex vivo* addition of BD GolgiStop™ to *in vitro*- or *in vivo*-stimulated cells blocks their intracellular transport processes. This results in the accumulation of most cytokine proteins in the Golgi complex and thereby enhances cytokine staining signals S*ee figures 2-4*). Enough BD GolgiStop reagent is provided for treating up to 1 liter of cell culture at a cell density of up to 2×10^6 cells/ml.

Kit components:

- Fixation/Permeabilization solution (125 ml)
- BD Perm/Wash™ Buffer, 10 × concentrate containing FBS* and saponin (dilute 1:10 in distilled H_20 prior to use) (100 ml)
- BD GolgiStop protein transport inhibitor containing monensin (also sold as a separate component; Cat. No. 554724) (0.7 ml)

Note: BD Perm/Wash™ buffer is supplied as 10× stock solution that contains FBS*. The color of this product may vary from lot-to-lot, and it may contain visible precipitate. Color variation and/or precipitate do not affect product performance.

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BD Cytofix/Cytoperm™ Plus Fixation/Permeabilization (BD GolgiPlug™ protein transport inhibitor) (Cat. No. 555028)

In addition to the Fixation/Permeabilization solution and BD Perm/Wash™ Buffer included in the BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit, this kit provides an alternative protein transport inhibitor, BD GolgiPlug™ containing brefeldin A. *See figures 2-4*. Sufficient BD GolgiPlug reagent is provided for treating up to 1 liter of cell culture at a cell density of up to 2×10^6 cells/ml.

Kit components:

- Fixation/Permeabilization solution (125 ml)
- BD Perm/Wash™ buffer, 10 × concentrate containing Fetal Bovine Serum (FBS)* and saponin (dilute 1:10 in distilled H_20 prior to use) (100 ml)
- BD GolgiPlug protein transport inhibitor containing brefeldin A (also sold as a separate component; Cat. No. 555029) (1 ml)

Note: BD Perm/Wash™ buffer is supplied as 10× stock solution that contains FBS*. The color of this product may vary from lot-to-lot, and it may contain visible precipitate. Color variation and/or precipitate do not affect product performance.

** Source of all serum proteins is from USDA inspected abattoirs located in the United States.*

Warnings and Precautions

All three kits described in this product manual contain Fixation/Permeabilization solution containing 4% paraformaldehyde, and either BD GolgiStop™ protein transport inhibitor containing 99.9% ethanol, or BD GolgiPlug™ protein transport inhibitor containing 99.9% dimethylsulfoxide and 0.1% brefeldin A. The following warnings and precautions apply to the aforementioned chemicals.

R 36/38	Irritating to eyes and skin
S3	Keep in a cool place
S26	In case of contact with eyes, rinse immediately with water and seek medical advise
S28	After contact with skin, wash immediately with plenty of water
R40	Limited evidence of a carcinogenic effect
S36/37	Wear suitable protective clothing and gloves

** Source of all serum proteins is from USDA inspected abattoirs located in the United States.*

General Procedure

A. Stimulation of Cells

Various *in vitro* methods have been reported for stimulating cytokine producing cells.[1-6] Polyclonal activators have been particularly useful for inducing and characterizing cytokine-producing cells. These include the following: phorbol esters plus calcium ionophore or ionomycin, phytohaemagglutinin, Staphylococcus enterotoxin B, and monoclonal antibodies directed against subunits of the TCR/CD3 complex (with or without antibodies directed against costimulatory receptors such as CD28).

Note: It has been reported that cell activation with PMA alone causes a transient loss of CD4 expression from the surface of mouse T cells. Cell activation with PMA and calcium ionophore together has been reported to cause a greater and more sustained decrease in CD4 expression, and also a decrease in CD8 expression in mouse thymocytes and mouse and human peripheral T lymphocytes.[8]

1. Procedure for Using BD GolgiStop™ Protein Transport Inhibitor (contains monensin)

Add 4 µl of **BD GolgiStop™** for every 6 ml of cell culture and mix thoroughly. It is recommended that **BD GolgiStop** not be kept in cell culture for longer than 12 hours.

Note: It is recommended that kinetic studies be performed to determine the optimal incubation time for each experimental system.

2. Procedure for Using BD GolgiPlug™ Protein Transport Inhibitor (contains Brefeldin A)

BD GolgiPlug™ contains DMSO which is a solid at 4°C. Be sure to thaw at room temperature prior to use. Add 1 µl of **BD GolgiPlug** for every 1 ml of cell culture and mix thoroughly. It is recommended that **BD GolgiPlug** not be kept in cell culture for longer than 12 hours.

Note: It is recommended that kinetic studies be performed to determine the optimal incubation time for each experimental system.

B. Protocol: Multicolor Staining for Cell Surface Antigens and Intracellular Cytokines

1. Harvest Cells

Viable cell populations can be prepared from *in vivo*-stimulated tissues or from *in vitro* stimulatory cultures treated with a protein transport inhibitor. The cells should be spun down out of the medium containing BD GolgiStop™ or BD GolgiPlug™, then resuspended in staining media, counted and transferred to plastic tubes or microwell plates for immunofluorescent staining. Cells should be protected from light throughout staining and storage.

2. Block Fc Receptors

Reagents that block Fc receptors may be useful for reducing nonspecific immunofluorescent staining.[7]

a. In the mouse system, purified 2.4G2 antibody, specific for FcγII/III receptors (BD Fc Block™; Cat. No. 553142), can be used to block nonspecific staining by fluorochrome-conjugated antibodies which is mediated by Fc receptors. To block mouse Fc receptors with Fc Block, preincubate cell suspension with 1 µg BD Fc Block/10^6 cells in 100 µl of Staining Buffer for15 minutes at 4°C. The cells can then be washed and stained with a fluorochrome-conjugated antibody specific for a cell surface antigen of interest which should be diluted appropriately in Staining Buffer.

b. Fc receptors on human cells can be pre-blocked by incubating cells with 10% normal human serum or an excess of irrelevant purified Ig from the same species and with the same isotype as the antibodies used for immunofluorescent staining.

3. Stain Cell Surface Antigens

a. Stain ~10^6 cells in 50 µl of Staining Buffer with the appropriate amount of a fluorochrome-conjugated monoclonal antibody specific for a cell surface antigen such as CD3, CD4, CD8, CD14, or CD19 (30 min, 4°C).

Note: Multicolor staining of different cell surface antigens can be done at this time to provide controls for setting proper compensation of the brightest fluorescent signals. Some antibodies which recognize cell surface markers may not bind to fixed/denatured antigen.[14] For this reason, it is recommended that the staining of cell surface antigens be done with live, unfixed cells PRIOR to fixation/permeabilization and staining of intracellular cytokines. Altering the procedure such that cells are fixed prior to staining of cell surface antigens requires that suitable antibody clones be empirically identified.

b. Wash cells 2 times with Staining Buffer (*e.g.*, 250 µl/wash for microwell plates, 1 ml/wash for tubes) and pellet by centrifugation (250 × g).

4. Fix and Permeabilize Cells

a. Thoroughly resuspend cells and add 100 µl per well for microwell plates (or 250 µl for tubes) of Fixation/Permeabilization solution for 20 min. at 4°C.

Note: Cell aggregation can be avoided by vortexing prior to the addition of the Fixation/Permeabilization solution.

b. Wash cells two times in 1× BD Perm/Wash™ buffer (*e.g.*, 1 ml/wash for staining in tubes and 250 µl/wash final volume for staining in microwell plates) and pellet.

Note: BD Perm/Wash™ buffer must be maintained in washing steps to keep cells permeabilized.

5. Alternative Fixation and Permeabilization Protocol

Cells can be fixed and stored to continue the intracellular staining at a later time.

a. Fixation and Storage of Cells

1. Resuspend cells in 100µl (or 1 ml/10^7 cells for bulk fixing) of a 4% paraformaldehyde solution at 4°C for 10-20 minutes.
2. Wash cells 2× in Staining Buffer.
3. Resuspend cells in Staining Buffer for storing cells at 4°C for up to 72 hrs or in 90% FCS/10% DMSO for storing at -80°C for longer periods of time.

b. Permeabilizing Fixed Cells.

1. For frozen cells, wash 2× to remove DMSO. For cells at 4°C, pellet and remove staining buffer.
2. Resuspend cells in BD Perm/Wash™ buffer for 15 minutes.
3. Pellet by centrifugation.
4. Stain for intracellular cytokines.

6. Stain for Intracellular Cytokines

a. Thoroughly resuspend fixed/permeabilized cells in 50 µl of BD Perm/Wash™ buffer containing a pre-determined optimal concentration of a fluorochrome-conjugated anti-cytokine antibody or appropriate negative control. Incubate at 4°C for 30 minutes in the dark.

b. Wash cells 2 times with 1× BD Perm/Wash™ buffer (1 ml/wash for staining in tubes and 250 µl/wash final volume for staining in microwell plates) and resuspend in Staining Buffer prior to flow cytometric analysis.

C. Flow Cytometric Analysis

Set PMT voltage and compensation using cell surface staining controls.
Set quadrant markers based on isotype or blocking controls and unstained cells.

Note: Frequencies of cytokine producing cells derived from activation of human PBMCs can vary widely for a particular cytokine, depending on the donor. Cryopreserved cells from one donor can be used for longitudinal studies.[2,6]

For proper flow cytometric analysis, cells stained by this method should be inspected by light microscopy and/or flow light scatter pattern to confirm that they are well dispersed. In order to make statistically significant population frequency measurements, sufficiently large sample sizes should be acquired during flow cytometric analysis. Bivariate dot plots or probability contour plots can be generated upon data reanalysis to display the frequencies of and patterns by which individual cells co-express certain levels of cell surface antigen and intracellular cytokine proteins.

D. Staining Controls

1. Positive Staining Controls

The Technical Data Sheets for BD Biosciences fluorochrome-conjugated anti-cytokine antibodies provide specific examples of *in vitro* culture systems which can induce detectable frequencies of cytokine-producing cells at specific time-points. Cells stimulated by these methods can be used as positive controls for experimental systems. Particularly important parameters for cell activation protocols include the use of protein transport inhibitors and the examination of multiple time points. Published reports of immunohistochemical staining[11-13] and ELISPOT analysis can also provide useful information regarding different experimental protocols for generating cytokine-producing cells.

2. Negative Staining Controls

The use of at least one of the following three controls is suggested to discriminate specific staining from artifactual staining. A combination of unstained cells and isotype control or blocking control is optimal. Investigators can choose which staining control best meets their research needs. Intracellular cytokine staining techniques and the use of blocking controls are described in detail by C. Prussin and D. Metcalf.[6]

a. Isotype control: Stain with an isotype-matched control of irrelevant specificity.

1. Resuspend cell pellet in 50 µl of BD Perm/Wash™ buffer containing a concentration of the isotype control antibody equal to that of the anti-cytokine antibody.
2. Incubate 30 min. at 4°C.
3. Wash cells using the aforementioned procedure for intracellular staining.

b. Ligand blocking control: Pre-block anti-cytokine antibody with recombinant cytokine.

1. Preincubate fluorochrome-labeled antibodies with cytokine diluted to the appropriate concentration in at least 50 µl of BD Perm/Wash™ buffer at 4°C for 30 minutes.
2. Resuspend fixed/permeabilized cells in 50 µl of pre-blocked labeled anti-cytokine antibody (in BD Perm/Wash™ buffer) and incubate 30 min. at 4°C.
3. Wash cells using the aforementioned procedure for intracellular staining.

c. Unlabeled antibody blocking control: Preincubate cells with unlabeled antibody.

1. Resuspend fixed/permeabilized cells in 25 µl BD Perm/Wash™ buffer containing unlabeled anti-cytokine antibody diluted to the appropriate concentration, and incubate 30 min. at 4°C .
2. After incubation, add fluorochrome-labeled anti-cytokine antibody at optimal concentration in 25µl BD Perm/Wash™ buffer (50 µl final volume) and incubate 30 min. at 4°C.
3. Wash cells using the aforementioned procedure for intracellular staining.

Solutions

Staining Buffer

- Dulbecco's PBS (DPBS) without Mg^{2+} or Ca^{2+}
- 1% heat-inactivated FCS
- 0.09% (w/v) sodium azide
- Adjust buffer pH to 7.4 - 7.6, filter (0.2 µm pore membrane), and store at 4°C

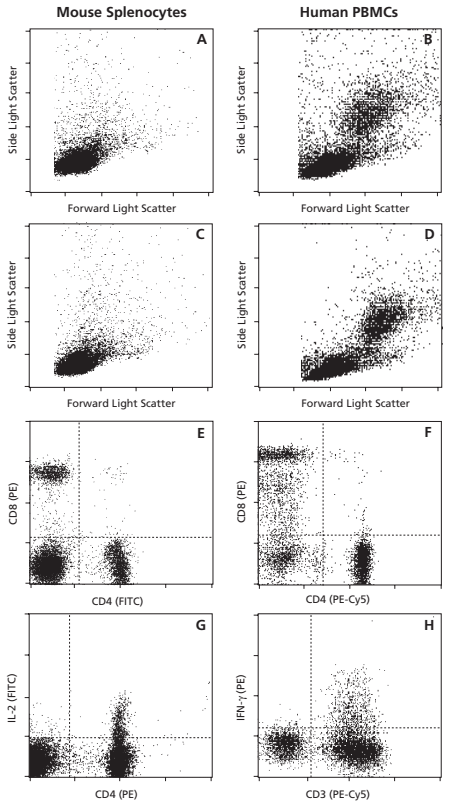


Figure 1. The effect of the BD Cytofix/Cytoperm solution on cell light scattering properties, cell surface antigen staining and intracellular cytokine staining.

Panels A and B show the forward light scatter and side light scatter profiles for fresh, untreated mouse splenocytes and Ficoll™-separated human peripheral blood mononuclear cells, respectively. Panels C and D show the forward light scatter and side light scatter profiles of the same cells (*in Panels A and B*) after they were treated with Fixation/Permeabilization solution. Panels E and F are examples of mouse and human cells, respectively, that were stained with anti-CD4 and anti-CD8 followed by incubation with the Fixation/Permeabilization solution. Panels G and H are examples of mouse and human cells, respectively, that were activated in culture in the presence of BD GolgiStop™ protein transport inhibitor, stained with PE-anti-CD4 or PE-Cy5 anti-CD3 followed by incubation with the Fixation/Permeabilization solution. The cells were then stained for intracellular IL-2 (mouse) and IFN-γ (human).

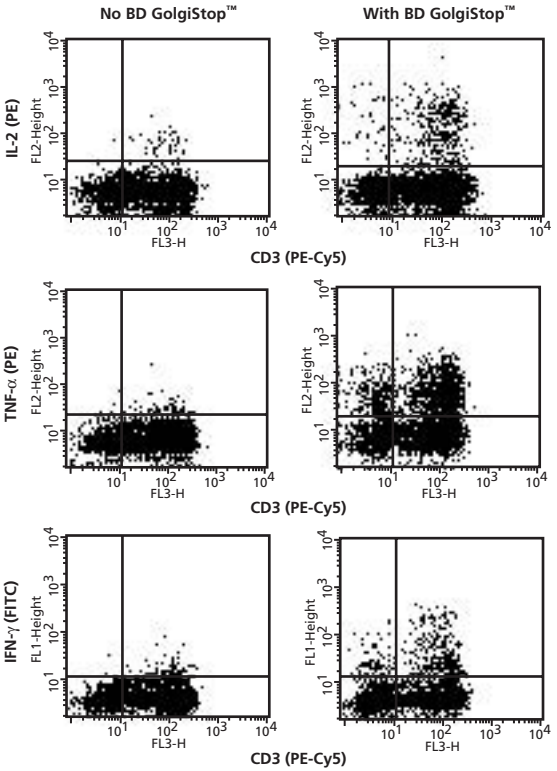


Figure 2. Effect of protein transport inhibitor on intracellular cytokine staining signal.

Human PMBCs were stimulated for 6 hours with PMA (50 ng/ml) and calcium ionophore A23187 (250 ng/ml), in the absence (*left panels*) or presence (*right panels*) of 2 µM monensin (aka BD GolgiStop™ protein transport inhibitor). Cells were harvested, stained with PE-Cy5 anti-human CD3 (Cat. No. 555334), fixed, permeabilized, and subsequently stained with PE anti-human IL-2 (Cat. No. 554566; *top panels*), PE anti-human TNF (Cat. No. 554513; *middle panels*), or FITC anti-human IFN-γ (Cat. No. 554551; *bottom panels*).

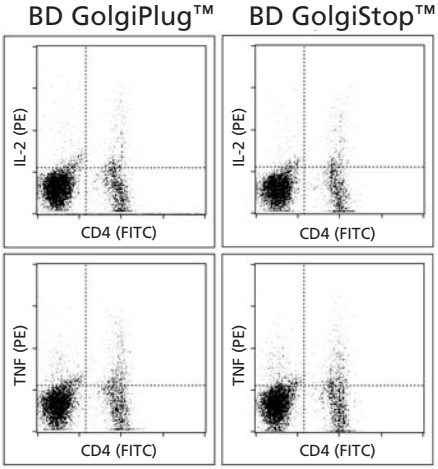
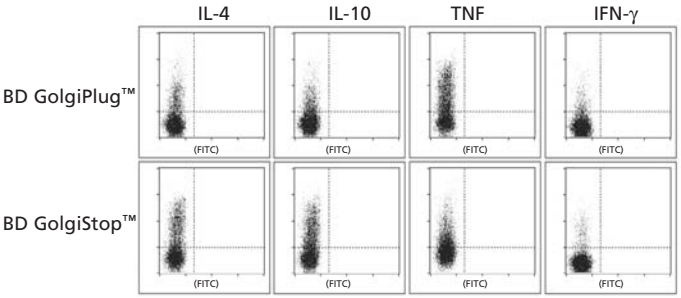


Figure 3. Comparison of the effects of BD GolgiPlug and BD GolgiStop on intracellular cytokine accumulation by activated mouse splenocytes.

IL-2 and TNF production was assessed by intracellular cytokine staining of mouse splenocytes that had been activated with immobilized anti-CD3 (25 µg/ml) + soluble anti-CD28 (2 µg/ml) for 4 hours in the presence of the protein transport inhibitor, BD GolgiPlug™ or BD GolgiStop™. In this case BD GolgiPlug and BD GolgiStop are comparably effective in inducing IL-2 and TNF accumulation.



	IL-2	IL-3	IL-4	IL-5	IL-10	GM-CSF	TNF	IFNγ
GolgiPlug™ 1 µg/ml	12.9	5.7	14.5	5.2	12.7	10.1	38.2	5.2
GolgiStop™ 2 µM	15.2	8.5	29.1	7.2	30.7	13.3	22.4	5.6

Figure 4. Comparison of the effects of BD GolgiPlug and BD GolgiStop on intracellular cytokine accumulation by re-activated purified mouse CD4+ cells.

Activated mouse CD4+ cells were restimulated with PMA (10 ng/ml) plus ionomycin (250 ng/ml) for 5 hours in the presence of BD GolgiPlug™ or BD GolgiStop™ and stained for the intracellular cytokines listed. In this case, BD GolgiPlug was more effective in inducing TNF accumulation, while BD GolgiStop was more effective in inducing IL-4 and IL-10 accumulation.

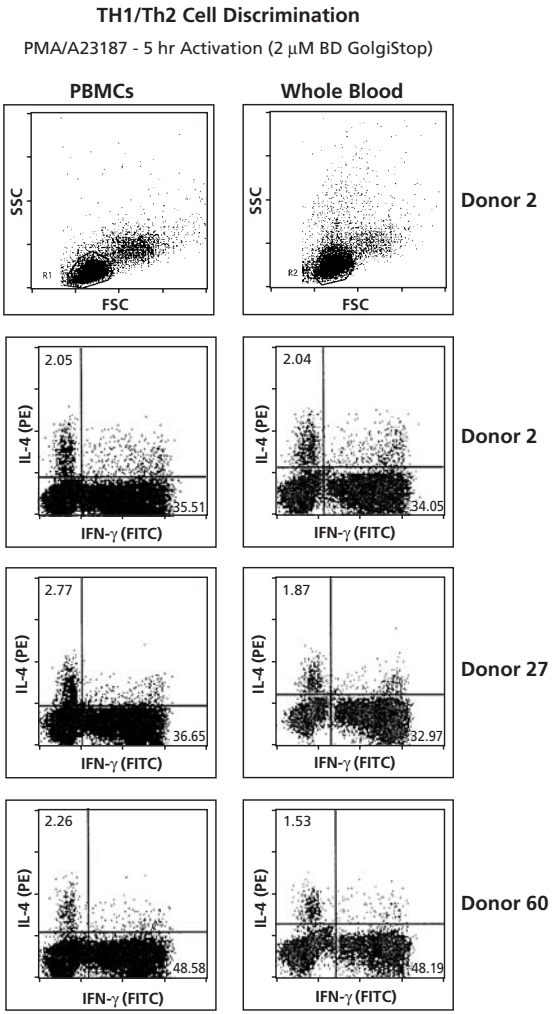


Figure 5. Frequencies of detectable cytokine-producing cells are comparable when staining activated PBMCs or activated whole blood from the same donor.

Ficoll-Hypaque™ purified human PBMCs (*left panels*) and whole blood (*right panels*) from each of three donors were activated with PMA (50 ng/ml) and ionophore A23187 (1 μg/ml) for 5 hr in the presence of 2 μM BD GolgiStop™, fixed, permeabilized and stained with PE-anti-human IL-4 (Cat. No. 554485; 0.06 μg) and FITC-anti-human IFN-γ (Cat. No. 554700; 0.25 μg), according to the BD Biosciences intracellular cytokine staining protocols (Standard or Whole Blood Method).

Alternative Protocol

Activation and Intracellular Staining of Whole Blood

1. Dilute whole blood 1:1 with IMDM. Mix well.
2. Add cell activator or mitogen to diluted blood (*e.g.*, 50 ng/ml PMA [Sigma, Cat. No. P-8139] + 1 µg/ml calcium ionophore A23187 [Sigma, Cat. No. C-7522] or PMA + 1 µM ionomycin [Sigma, Cat. No. I-0634] - final concentration).
3. Add protein transport inhibitor:
   - BD GolgiPlug™ protein trasnport inhibitor, (Cat. No. 555029) 1 µl solution / 1 ml of diluted blood (aka Brefeldin A, 1.0 µg/ml final concentration) or
   - BD GolgiStop™ protein trasnport inhibitor, (Cat. No. 554724) 0.7 µl solution / 1 ml of diluted blood (aka Monensin, 2.0 µM final concentration).
4. Vortex briefly to mix. Aliquot into 12 × 75 mm plastic tubes, 200 µl/tube. Incubate for 4-6 hrs in 5% CO_2 at 37°C. Optimal incubation times must be investigated and incubation time should not exceed 24 hours.
5. Add 2 ml 1× BD PharmLyse™ (Cat. No. 555899), vortex, incubate 10 min at RT in the dark.
6. Spin 5 min, 500 × g.
7. Aspirate supernatant. Wash 1× in Staining Buffer. Spin 5 min at 500 × g. Aspirate supernatant.
8. Resuspend cell pellet in 100 µl of Staining Buffer containing an optimal concentration of fluorochrome-conjugated antibodies specific for cell surface antigens (*e.g.*, FITC-, PE-, PE-Cy5- anti- CD3, CD4, CD8, CD14, etc.). Incubate for 15 min at RT in dark. Wash 1× in Staining Buffer. Spin 5 min, 500 × g. Aspirate supernatant.
9. Fix and permeabilize cells by adding 500 µl of Fixation/Permeabilization solution. Vortex and incubate at RT in the dark for 20 min. Spin 5 min, 500 × g. Aspirate supernatant.
10. Wash cells by adding 2 ml BD Perm/Wash™ buffer. Incubate for 10 min. at RT in the dark. Spin 5 min, 500 × g. Aspirate supernatant.
11. Resuspend cell pellet in 100 µl BD Perm/Wash™ buffer containing an optimal concentration of fluorochrome-conjugated anti-cytokine antibody for intracellular staining (*e.g.*, ≤ 0.25 µg/test). See technical data sheet for antibody-specific recommended concentrations. Stain for 30 min at RT in the dark.
12. Wash cells by adding 2 ml BD Perm/Wash™ buffer. Spin 5 min, 500 × g. Aspirate supernatant.
13. Resuspend cell pellet in 500 µl PBS/2% paraformaldehyde solution (vortex while adding PBS/PFA).
14. Analyze by flow cytometry.

Expected results:

- 5 hr PMA/Ionomycin activation of normal human blood will generally yield detectable frequencies of IL-2, IL-4, IFN-γ, and TNF expressing cells (lymphocyte gate).
- 6 hr LPS activation of normal human blood will generally yield detectable frequencies of IL-1α, IL-6, IL-8, and MIP-1α expressing cells (monocyte gate).

Note: There is variation in typical cytokine - producer cell frequency amongst different donors.

Solutions

Staining Buffer

- Dulbecco's PBS (DPBS) without Mg^{2+} or Ca^{2+}
- 1% heat-inactivated FCS
- 0.09% (w/v) sodium azide
- Adjust buffer pH to 7.4 - 7.6, filter (0.2 µm pore membrane), and store at 4°C

IMDM (BioWhittaker - Cat. No. 12-726Q) is a cell culture medium beneficial in maintaining cell viability of whole blood in culture.

The following are anti-cytokine antibody clones that were selected and screened for compatibility with the described intracellular staining procedure.

Cytokine Reagents for Immunofluorescence Staining and Flow Cytometric Analysis

1. FITC and PE labeled anti-cytokine antibodies for staining intracellular cytokines.* Unlabeled antibody and recombinant cytokine blocking controls.

			Catalog Number							
			Staining Antibodies						Blocking Controls	
Specificity	Clone	Isotype	FITC	PE	APC	Alexa Fluor® 488	Alexa Fluor® 647	PE-Cy7	Unlabeled	Recombinant Cytokine
Human										
IL-2	MQ1-17H12	Rat IgG_{2a}	554565	554566	554567				554563	554603
IL-3	BVD3-1F9	Rat IgG_1		554676		557727	557738		554673	554604
IL-4	MP4-25D2	Rat IgG_1	554484	554485	554486	557990	557905	557989	554486	
IL-4	8D4-8	Mouse IgG_1		554516					556917	554605
IL-5	TRFK5	Rat IgG_1		554395	554396				554392	554606
IL-5	JES1-39D10	Rat IgG_{2a}		554489					554487	554606
IL-6	MQ2-13A5	Rat IgG_1	554544	554545					554542	550071
IL-6	MQ2-6A3	Rat IgG_{2a}	554696	554697					554694	550071
IL-8	G265-8	Mouse IgG_{2b}	554719	554720					554717	554609
IL-10	JES3-9D7	Rat IgG_1		554498					554496	554611
IL-10	JES3-19F1	Rat IgG_{2a}		554706	554707		557902		554704	554611
IL-12 (p40/p70)	C11.5.14	Mouse IgG_1	554574	554575	554576				554573	
IL-12 (p70)	20C2	Rat IgG_1		557020					557018	
IL-13	JES10-5A2	Rat IgG_1		554571					554569	554614
IL-16	14.1	Mouse IgG_{2a}		554736					554734	554637
GM-CSF	BVD2-21C11	Rat IgG_{2a}	554506	554507					554503	550068
GRO	10G4	Mouse IgG_1		555042					555041	
IFN-γ	B27	Mouse IgG_1	554700	554701	554702	557718	557729	557643	554699	
IFN-γ	4S.B3	Mouse IgG_1	554551	554552		557866	557904	557844	554549	
IP-10	6D4/D6/G2	Mouse IgG_{2a}		555049					556886	
MCP-1	5D3-F7	Mouse IgG_1	554665	554666					554662	
MCP-3	9H11	Mouse IgG_1		555033					555031	
MIG	B8-11	Mouse IgG_1		555039					556918	
RANTES	2D5	Mouse IgG_1		554732					556859	
TNF	MAb11	Mouse IgG_1	554512	554513	554514	557722	557733	557647	554510	554618
LT-α	359-81-11	Mouse IgG_1		554556					554554	554619
Mouse										
IL-2	JES6-5H4 Rat	IgG_{2b}	554427	554428	554429	557725	557736		554425	550069
IL-3	MP2-8F8	Rat IgG_1		554383					554380	554579
IL-4	BVD4-1D11	Rat IgG_{2b}	554388	554389			557970		554386	550067
IL-4	11B11	Rat IgG_1		554435	554436	557728	557739		554433	550067
IL-5	TRFK5	Rat IgG_1		554395	554396				554392	554581
IL-6	MP5-20F3	Rat IgG_1		554401					554399	554582
IL-10	JES5-16E3	Rat IgG_{2b}	554466	554467	554468				554464	550070
IL-12 (p40/p70)	C15.6	Rat IgG_1		554479	554480	557972	557977		554477	
IL-17	TC11-18H10	Rat IgG_1		559502					559501	
GM-CSF	MP1-22E9	Rat IgG_{2a}		554406					556916	554586
IFN-γ	XMG1.2	Rat IgG_1	554411	554412	554413	557724	557735	557649	554409	
MCP-1	2H5	Hamster IgG		554443					554441	554590
TNF	MP6-XT22	Rat IgG_1	554418	554419	554420	557719	557730	557644	554416	554589
TNF	TN3-19.12	Hamster IgG		559503					559500	554589
Rat										
IL-4	OX-81	Mouse IgG_1		555082					555079	555107
IL-10	A5-4	Mouse IgG_{2b}		555088					555087	555113
GM-CSF	B61-5	Mouse IgG_1		555092					556885	555111
IFN-γ	DB-1	Mouse IgG_1	559498	559499					559650	
MCP-1	2H5	Hamster IgG		554443					554441	555110
TNF	TN3-19.12	Hamster IgG		559503					559500	555109

Cytokine Reagents for Immunofluorescence Staining and Flow Cytometric Analysis

Intracellular Cytokine-Positive Control Cells

Description	Cytokines Expressed	Cat. No.
Human		
HiCK 1	IL-2, IFN-γ, TNF	555061
HiCK 2	IL-3, IL-4, IL-10, IL-13, GM-CSF	555062
HiCK 3	IL-1-α, IL-1β, IL-6, IL-12, TNF	555063
HiCK 4	IL-8, GROα, IP-10, MCP-1, MCP-3, MIG, MIP-1α	555064
Mouse		
MiCK 1	IL-2, IFN-γ, TNF	554652
MiCK 2	IL-4, IL-10, GM-CSF, TCA3	554653
MiCK 3	IL-1α, IL-6, IL-12. MCP-1, TNF	554654
Rat		
RiCK 2	IL-4, IL-10, GM-CSF	555094

Isotype Controls

		Catalog Number					
Description	Clone	FITC	PE	APC	Alexa Fluor® 488	Alexa Fluor® 647	PE-Cy7
Mouse IgG_1 isotype control	MOPC-21	554679	554680	554681	557721	557732	557646
Mouse IgG_{2a} isotype control	G155-178	554647			557703	557715	
Mouse IgG_{2b} isotype control	27-35	555057	555058				
Rat IgG_1 isotype control	R3-34	554684	554685	554686	557720	557731	557645
Rat IgG_{2a} isotype control	R35-95	554688	554689	554690		552690	
Rat IgG_{2b} isotype control	A95-1	556923	556925	556924	557726	557677	
Hamster IgG_1, λ isotype control	G235-2356		554711				

Disclaimers:

1. Use of these products to measure activation antigens expressed on mononuclear cell subsets for the purpose of monitoring immunoregulatory status can fall under one or more claims of the following patents: US Patent Nos. 5,445,939, 5,656,446, 5,843,689; European Patent No. 319,543; Canadian Patent No. 1,296,622; Australian Patent No. 615,880; and Japanese Patent No. 2,769,156.
2. The following products are covered by the listed patents held or licensed by Becton Dickinson and Company. Phycoerythin (PE) conjugates and Allophycocyanin (APC). US Patent No. 4,520,110 and 4,524,104; European Patent No. 0076695; Canadian Patent No. 1,179,942.

References:

1. Assenmacher, M., J. Schmitz and A. Radbruch. 1994. Flow cytometric determination of cytokines in activated murine T helper lymphocytes: Expression of interleukin-10 in interferon-γ and in interleukin-4-expressing cells. *Eur. J. Immunol.* 24:1097-1101.
2. Elson, L. H., T. B. Nutman, D. D. Metcalfe and C. Prussin. 1995. Flow cytometric analysis for cytokine production identifies Th1, Th2, and Th0 cells within the human $CD4^+$ CD27- lymphocyte subpopulation. *J. Immunol.* 154:4294-4301.
3. Sander, B., J. Andersson and U. Andersson. 1991. Assessment of cytokines by immunofluorescence and the paraformaldehyde-saponin procedure. *Immunol. Rev.* 119:65-93.
4. Vikingson, A., K. Pederson and D. Muller. 1994. Enumeration of IFN-γ producing lymphocytes by flow cytometry and correlation with quantitative measurement of IFN- γ. *J. Immunol. Meth.* 173:219-228.
5. Jung, T., U. Schauer, C. Heusser, C. Neumann and C. Rieger. 1993. Detection of intracellular cytokines by flow cytometry. *J. Immunol. Meth.* 159:197-207.
6. Prussin, C. and D. Metcalfe. 1995. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J. Immunol. Meth.* 188: 117-128.
7. Ferrick, D. A., M. D. Schrenzel, T. Mulvania, B. Hsieh, W. G. Ferlin and H. Lepper. 1995. Differential production of interferon-g and interleukin-4 in response to Th1- and Th2-stimulating pathogens by γδ T cells *in vivo*. *Nature.* 373:255-257.
8. Andersson, S. and C. Coleclough. 1993. Regulation of CD4 and CD8 expression on mouse T cells. *J. Immunol.* 151: 5123-5134.
9. Andersson, U. and J. Andersson. 1994. Immunolabelling of cytokine producing cells in tissues and suspension. In *Cytokine Producing Cells,* eds. D. Fradelizie and D. Emelie. INSERM, Paris. p. 32-49.
10. Litton, M., B. Sander, E. Murphy, A. O'Garra, and J. Abrams. 1994. Early expression of cytokines in lymph nodes after treatment *in vivo* with Staphylococcus enterotoxin B. *J. Immunol. Meth.* 175: 47-58.
11. Sander, B., I. Hoiden, U. Andersson, E. Moller, and J. Abrams. 1993. Similar frequencies and kinetics of cytokine producing cells in murine peripheral blood and spleen. *J. Immunol. Meth.* 166: 201-214.
12. Andersson, J., J. Abrams, L. Bjork, K. Funa, M., Liton, K. Agren, and U. Andersson. 1994. Concomitant in vivo production of 19 dif ferent cytokines in human tonsils. *Immunol.* 83: 16-24.
13. Litton, M., J. Andersson, L. Bjork, T. Fehniger, A-K. Ulfgren, and U. Andersson. 1996. Cytoplasmic cytokine staining in individual cells. In *Human Cytokine Protocols,* eds. Debets and Savelkoul. *Humana Press.*
14. Alaverdi, N. and J.B. Waters. Enhanced detection of antigens by flow cytometry with the BD Cytofix/Cytoperm™ intracellular staining kit. *Hotlines.* 3:6, 7, 15, and 16.

Notes

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