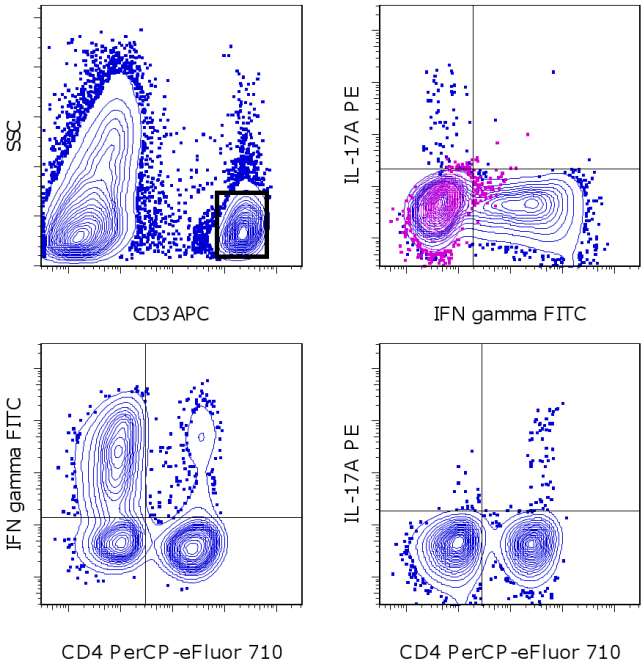
eZKine™ Th1/Th17 Whole Blood Intracellular Cytokine Kit

Catalog Number: 8822-6850

RUO: For Research Use Only. Not for use in diagnostic procedures.



Staining of human whole blood with the eZKine™ Th1/Th17 Whole Blood Intracellular Cytokine Kit. Freshly isolated whole blood was stimulated for 5 hours with Cell Stimulation Cocktail (plus protein transport inhibitors) (500X) (cat. 00-4975). After stimulation, samples were fixed and lysed with eZKine Fix/Lyse buffer, washed with Permeabilization Buffer and stained with the Th1/Th17 Cocktail. Total cells were gated for CD3 APC staining (top left) and then analyzed for staining of CD4 PerCP-eFluor® 710, IFN gamma FITC, and IL-17A PE as indicated. Staining of the Isotype Control Cocktail A is indicated in pink (top right).

Product Information

Contents: eZKine™ Th1/Th17 Whole Blood Intracellular Cytokine Kit

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Handling Conditions: Use within 6 months of opening or by date indicated on the bottle

Temperature Limitation: Store at 2-8°C. Do not freeze. Light-sensitive material.

LOT **Batch Code:** Refer to vial

Use By: Refer to vial

Contains sodium azide and formaldehyde

Description

This eZKine Th1/Th17 Whole Blood Intracellular Cytokine Kit is designed to rapidly identify cytokine producing T lymphocytes of the Th1 and Th17 lineages after stimulation of whole peripheral blood samples. Stimulation of whole blood in the presence of Protein Transport Inhibitor Cocktail (cat. 00-4980, not included) is followed by red blood cell lysis and fixation in a single step and subsequent permeabilization. Samples are then ready to stain with the Th1/Th17 cocktail and concentration-matched Isotype Control cocktail.

Th1 cells are a CD4+ T cell subset that plays an important role in host defense against intracellular bacteria and viruses. Th1 lineage commitment is controlled by the transcription factor, T-bet, and these cells are a primary source of IFN gamma, which stimulates macrophages, lymphocytes, and PMNs in the destruction of bacterial pathogens. IFN gamma also helps foster the development of cytotoxic lymphocytes (CTL & NK cells) that are responsible for the cell-mediated immune response against viruses and tumor cells. Dysregulation of the Th1 response plays a critical role in many inflammatory and autoimmune diseases.

Th17 cells play a key role in barrier defense against extracellular pathogens such as bacteria and fungi and play a significant role in autoimmune disease. They are defined by their expression of the transcription factor ROR gamma t and the inflammatory cytokine IL-17A. Through their activation and subsequent cytokine production, Th17 trigger pro-inflammatory signaling that promotes neutrophil mobilization and the expression of antimicrobial peptides. Some plasticity between the Th1 and Th17 lineages has been reported. Furthermore, a pathogenic Th17 subpopulation has

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been described with expression of both IL-17 and IFN gamma, as well as ROR gamma t and T-bet. These cells are implicated in several inflammatory and autoimmune diseases.

Components
eZKine Th1/Th17 Cocktail (cat. 22-7780-71): 25 tests. Store at 2-8°C. This cocktail contains the following antibodies:
Anti-Human CD3 (SK7) APC
Anti-Human CD4 (SK3) PerCP-eFluor® 710
Anti-Human IL-17A (eBio64DEC17) PE
Anti-Human IFN gamma (4S.B3) FITC
eZKine Isotype Control Cocktail A (cat. 22-7781-71): 25 tests. Store at 2-8°C. This cocktail contains the following antibodies:
Anti-Human CD3 (SK7) APC
Anti-Human CD4 (SK3) PerCP-eFluor® 710
Mouse IgG1 K Isotype Control PE
Mouse IgG1 K Isotype Control FITC
eZKine Fix/Lyse Concentrate (4X) : 15 mL. Store at 2-8°C. Avoid agitation.
eZKine Fix/Lyse Diluent: 50 mL. Store at 2-8°C.
Permeabilization Buffer (10X): 20 mL. Store at 2-8°C. *Note: The Permeabilization Buffer (10X) has a natural tendency to precipitate, however, its function is not affected by this. To clarify, the solution can be filtered after dilution to 1X working solution.*

Applications Reported
The eZKine Th1/Th17 Whole Blood Intracellular Cytokine Kit has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested
The eZKine Th1/Th17 Whole Blood Intracellular Cytokine Kit has been pre-titrated and tested by intracellular staining and flow cytometric analysis of stimulated human whole blood following the eZKine protocol. The Th1/Th17 Cocktail and Isotype Control Cocktail can be used at 20 µL per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10^5 to 10^8 cells/test.

References
Cosmi L, Maggi L, Santarlasci V, Liotta F, Annunziato F. T helper cells plasticity in inflammation. Cytometry A. 2013 Sep 5.

Shi G, Vistica BP, Nugent LF, Tan C, Wawrousek EF, Klinman DM, Gery I. Differential involvement of Th1 and Th17 in pathogenic autoimmune processes triggered by different TLR ligands. J Immunol. 2013 Jul 1;191(1):415-23.

Duhen R, Glatigny S, Arbelaez CA, Blair TC, Oukka M, Bettelli E. Cutting edge: the pathogenicity of IFN-gamma-producing Th17 cells is independent of T-bet. J Immunol. 2013 May 1;190(9):4478-82.

Lee YK, Turner H, Maynard CL, Oliver JR, Chen D, Elson CO, Weaver CT. Late developmental plasticity in the T helper 17 lineage. Immunity. 2009 Jan;30(1):92-107.

Bettelli E, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of T(H)17 cells. Nature. 2008 Jun 19;453(7198):1051-7.

Related Products
00-4975 eBioscience™ Cell Stimulation Cocktail (plus protein transport inhibitors) (500X)

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00-4980 eBioscience™ Protein Transport Inhibitor Cocktail (500X)
01-1111 OneComp™ eBeads Compensation Beads
11-7319 eBioscience™ Anti-Human IFN gamma FITC (4S.B3)
12-7179 eBioscience™ Anti-Human IL-17A PE (eBio64DEC17)
16-0289 eBioscience™ Anti-Human CD28 Functional Grade Purified (CD28.2)
16-0499 eBioscience™ Anti-Human CD49d (Integrin alpha 4) Functional Grade Purified (9F10)
17-0036 eBioscience™ Anti-Human CD3 APC (SK7)
46-0047 eBioscience™ Anti-Human CD4 PerCP-eFluor™ 710 (SK3 (SK-3))
8822-6856 eZKine™ 4-Color Compensation Control Kit

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invitrogen

eZKine™ Whole Blood Intracellular Cytokine Staining Kit

Protocol 1: eZKine™ Whole Blood Intracellular Cytokine Staining

Materials Provided

- Refer to the components section of the datasheet.

Other Materials Needed

- 12 x 75 mm round bottom test tubes
- Flow Cytometry Staining Buffer (cat. 00-4222)
- [Optional] Cell Stimulation Cocktail (plus protein transport inhibitors) (500X) (cat. 00-4975)
- [Optional] Protein Transport Inhibitor Cocktail (500X) (cat. 00-4980)

Time Requirements

- Blood stimulation, 4-6 hours recommended
- Fixation/lysis, 25 minutes (or up to 3 days)
- Permeabilization, 10 minutes
- Staining, 30 minutes
- Final washing, 5-10 minutes

Experimental Procedure

Stimulation of Whole Blood

1. Use blood collected with sodium heparin anticoagulant. Other anticoagulants, such as sodium citrate and EDTA, may interfere with lymphocyte activation.
2. Split blood into 2 separate sterile conical tubes. Label one for the stimulation and the other for the unstimulated control.
    - Generally, calculate the volume of blood to stimulate based on 100 µL of blood per stained sample specified in the experimental design.
    - Volumes of blood larger than 5 mL per 15 mL conical tube have not been tested.
3. Stimulation:
    - Add Cell Stimulation Cocktail plus Protein Transport Inhibitors (cat. 00-4975) at 1:500 directly to the blood in the stimulation tube.
    - Alternatively, add stimulant(s) as specified by a given experimental design. Be sure to add Protein Transport Inhibitor Cocktail (cat. 00-4980) to these stimulations as well.
    - Add Protein Transport Inhibitor Cocktail (cat. 00-4980) at 1:500 directly to the blood in the unstimulated tube.
4. Incubate for 4-6 hours in a 37°C incubator with 5% CO_2. Loosen the cap on the tube to allow for gas exchange.
    - Alternative incubation times may be appropriate as specified by the experimental design.

Lysis, Fixation, Permeabilization and Staining of Blood

5. Prepare buffers:
    - Prepare fresh eZKine Fix/Lyse working solution by diluting eZKine Fix/Lyse Concentrate (1 part) with eZKine Fix/Lyse Diluent (3 parts). You will need 1 mL of the Fixation/Permeabilization working solution for each sample.
    - Prepare a 1X working solution of Permeabilization Buffer by diluting 10X Permeabilization Buffer with distilled water prior to use. You will need ~6 mL of 1X Permeabilization Buffer for each sample.
6. Aliquot 100 µL of stimulated or unstimulated blood per 12 x 75 mm round bottom tube.
7. Add 1 mL of freshly-prepared eZKine Fix/Lyse Buffer and incubate for 25 minutes to 2 hours at room temperature or up to 3 days at 2-8°C.
8. Add 2 mL of 1X Permeabilization Buffer and centrifuge at 600 x *g* at room temperature for 4 minutes. Discard the supernatant.
9. Add 2 mL of 1X Permeabilization Buffer and centrifuge at 600 x *g* at room temperature for 4 minutes. Discard the supernatant and pulse vortex the sample to completely dissociate the pellet.
10. Add 20 µL of the staining cocktail or the isotype control cocktail to the appropriate tubes and incubate at room temperature for 30 minutes while protecting from light.
11. Add 2 mL 1X Permeabilization Buffer and centrifuge at 600 x *g* at room temperature for 4 minutes. Discard the supernatant.

12. Add 2 mL of Flow Cytometry Staining Buffer and centrifuge at 600 x *g* at room temperature for 4 minutes. Discard the supernatant.
13. Resuspend stained cells in an appropriate volume of Flow Cytometry Staining Buffer. Store at 2-8°C while protecting from light until ready to analyze on a flow cytometer.
    - For storage of up to 3 days prior to analysis, we recommend adding 100 µL of IC Fixation Buffer (cat. 00-8222) to the residual volume in the tube (typically ~100 µL of cells). Store at 2-8°C while protecting from light until ready to analyze on a flow cytometer.

Protocol 2: eZKine™ Whole Blood Intracellular Cytokine Staining in 96-well plates

Materials Provided

- Refer to the components section of the datasheet.

Other Materials Needed

- 96-well tissue culture plates
- Flow Cytometry Staining Buffer (cat. 00-4222)
- [Optional] Cell Stimulation Cocktail (plus protein transport inhibitors) (500X) (cat. 00-4975)
- [Optional] Protein Transport Inhibitor Cocktail (500X) (cat. 00-4980)

Time Requirements

- Blood stimulation, 4-6 hours recommended
- Fixation/lysis, 25 minutes (or up to 3 days)
- Permeabilization, 15 minutes
- Staining, 30 minutes
- Final washing, 5-10 minutes

Experimental Procedure

Stimulation of Whole Blood

1. Use blood collected with sodium heparin anticoagulant. Other anticoagulants, such as sodium citrate and EDTA, may interfere with lymphocyte activation.
2. Pipet 25 µL of whole blood into the wells of a 96-well plate.
    - A 10-fold dilution of whole blood with eZKine Fix/Lyse buffer is necessary for complete lysis. If using a standard 96-well plate, 25 µL of blood is the maximum recommended to accommodate 250 µL of eZKine Fix/Lyse buffer used in Step 6.
3. Stimulation:
    - Add Cell Stimulation Cocktail plus Protein Transport Inhibitors (cat. 00-4975) at 1:500 directly to the blood in the appropriate well(s).
    - Alternatively, add stimulant(s) as specified by a given experimental design. Be sure to add Protein Transport Inhibitor Cocktail (cat. 00-4980) to these stimulations as well.
    - Add Protein Transport Inhibitor Cocktail (cat. 00-4980) at 1:500 directly to the blood in the unstimulated control well(s).
4. Cover the plate and incubate for 4-6 hours in a 37°C incubator with 5% CO_2.
    - Alternative incubation times may be appropriate as specified by the experimental design.

Lysis, Fixation, Permeabilization and Staining of Blood

5. Prepare buffers:
    - Prepare fresh eZKine Fix/Lyse working solution by diluting eZKine Fix/Lyse Concentrate (1 part) with eZKine Fix/Lyse Diluent (3 parts). You will need 250 µL of the Fixation/Permeabilization working solution for each well (approximately 25 mL per plate).
    - Prepare a 1X working solution of Permeabilization Buffer by diluting 10X Permeabilization Buffer with distilled water prior to use. You will need approximately 1 mL of 1X Permeabilization Buffer for each well (approximately 100 mL per plate).
6. Add 250 µL of freshly-prepared eZKine Fix/Lyse Buffer to each well and incubate for 25 minutes to 2 hours at room temperature or up to 3 days at 2-8°C.
7. Centrifuge at 600 x *g* at room temperature for 4 minutes. Decant or aspirate the supernatant.
8. Add 250 µL of 1X Permeabilization Buffer to each well and centrifuge at 600 x *g* at room temperature for 4 minutes. Decant or aspirate the supernatant.
9. Add 250 µL of 1X Permeabilization Buffer to each well and centrifuge at 600 x *g* at room temperature for 4 minutes. Decant or aspirate the supernatant and pulse vortex the sample to completely dissociate the pellet.

10. Add 100 µL of 1X Permeabilization Buffer to each well.
11. Add 20 µL of the staining cocktail or the isotype control cocktail to the appropriate wells and incubate at room temperature for 30 minutes while protecting from light.
12. Add 250 µL of 1X Permeabilization Buffer and centrifuge at 600 x *g* at room temperature for 4 minutes. Decant or aspirate the supernatant.
13. Add 250 µL of Flow Cytometry Staining Buffer and centrifuge at 600 x *g* for 4 minutes. Decant or aspirate the supernatant.
14. Resuspend stained cells in an appropriate volume (100-200 µL) of Flow Cytometry Staining Buffer. Store at 2-8°C while protecting from light until ready to analyze on a flow cytometer.
    - For storage of up to 3 days prior to analysis, we recommend adding 100 µL of IC Fixation Buffer (cat. 00-8222) to the residual volume in each well. Store at 2-8°C while protecting from light until ready to analyze on a flow cytometer.

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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