

Intracelulární značení cytokinů

Materiál

00-8222-49 eBioscience IC Fixation Buffer 125 ml +
00-8333-56 eBioscience Permeabilization Buffer(10x) 100 ml

nebo

88-8823-88 eBioscience Fixation & Permeabilization Kit

OBSAHUJE :

- 1. Brefeldin A roztok:** 1ml. Brefeldin A je dodáván 1000x koncentrovaný. Brefeldin A je inhibitor transportu proteinů a inkubace buněk s tímto inhibitorem během aktivace vede k akumulaci cytokinů v Golgiho aparátu, což zvyšuje efektivitu výsledného značení.
- 2. IC Fixační Pufr:** 125ml. Tento pufr je dodáván v pracovní koncentraci. Obsahuje 4% Paraformaldehyd.
- 3. Permeabilizační Pufr:** 100ml. Permeabilizační pufr je dodáván 10x koncentrován. 1x Permeabilizační pufr obsahuje 0.1% saponin (detergent) a 0.09% azid sodný.

00-4509-51 eBioscience Cytokine IC Control Cells for Human 1 ml

„The Human Cytokine Positive Control Cells” jsou doporučenou pozitivní kontrolou pro intracelulární stanovení cytokinů. Buňky periferní krve byly stimulované po dobu dvou dní immobilizovanou anti-human CD3 (10 µg/mL), rozpustným anti-human CD28 (2 µg/mL), a rekombinantním IL-2 (10 ng/mL) a IL-4 (20 ng/mL), a následně tři další dny s IL-2 a IL-4. Poté byly buňky nestimulovány PMA (5 ng/ml) a ionomycinem (500 ng/ml) v přítomnosti Brefeldin A po dobu 4 hodin. Tyto kontrolní buňky zahrnují subpopulace exprimující IL-2, IL-4, IL-13, IFN γ , a GM-CSF. Buňky byly fixovány formaldehydem a jsou nesuspendované ve stabilizačním pufru, takže při -80°C mohou být skladovány až 6 měsíců a při 2-8°C až měsíc.

PROTOKOL

1. Nejprve naznačit buněčné povrchy standardním postupem (viz. Extracelulární značení) – POKUD JE TŘEBA.
2. Po promytí pipetovat k peletě ($1-2 \times 10^6$ buněk) 100 μ l IC Fixation Buffer naředěného na pracovní koncentraci roztoku a inkubovat 20min. při 4°C
3. 2x promýt 200 μ l roztokem Permeabilization Buffer (permeabilizační roztok), centrifugovat (500-600g, 2min., 4°C) a opatrně odsát maximum tekutiny.
4. Protilátku ředěnou v Permeabilization Buffer roztoku nanést na buňky (10 μ l/jamku) a inkubovat 30min., 4°C, ve tmě
5. 2x promýt ve 200 μ l Permeabilization Buffer roztoku.
6. Resuspendovat v požadovaném množství PBS (50-100 μ l) a pokud je třeba, přenést do mikroskopavek .

POZNAMKA – kontrolní buňky jsou fixovány a permeabilizovány, takže stačí je jen 2x promýt v 200 μ l Permeabilization Buffer roztoku a přejít rovnou do kroku 4 – značení protilátkou.

Dostupné eBio protilátky proti cytokinům a chemokinům

anti-Human

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| 11-7118 | Anti-Human IL-1 alpha FITC |
| 11-7015 | Anti-Human IL-1 Receptor Antagonist FITC |
| 11-7018 | Anti-Human IL-1 beta FITC |
| 12-7018 | Anti-Human IL-1 beta PE |
| 11-7019 | Anti-Human IL-1 alpha FITC |
| 46-7029 | Anti-Human IL-2 PerCP-eFluor® 710 |
| 11-7029 | Anti-Human IL-2 FITC |
| 12-7029 | Anti-Human IL-2 PE |
| 17-7029 | Anti-Human IL-2 APC |
| 14-7048 | Anti-Human IL-4 Purified |
| 12-7049 | Anti-Human IL-4 PE |
| 17-7049 | Anti-Human IL-4 APC |
| 25-7049 | Anti-Human IL-4 PE-Cy7 |
| 53-7049 | Anti-Human IL-4 Alexa Fluor® 488 |
| 12-7052 | Anti-Human/Mouse IL-5 PE |
| 11-7069 | Anti-Human IL-6 FITC |
| 12-7069 | Anti-Human IL-6 PE |
| 56-7069 | Anti-Human IL-6 Alexa Fluor® 700 |
| 51-7097 | Anti-Human IL-9 Alexa Fluor® 647 |
| 12-7098 | Anti-Human IL-9 PE |
| 12-7108 | Anti-Human IL-10 PE |
| 51-7108 | Anti-Human IL-10 Alexa Fluor® 647 |
| 53-7108 | Anti-Human IL-10 Alexa Fluor® 488 |
| 48-7129 | Anti-Human IL-12/IL-23 p40/p70 eFluor® 450 |
| 12-7129 | Anti-Human IL-12/IL-23 p40/p70 PE |
| 51-7129 | Anti-Human IL-12/IL-23 p40/p70 Alexa Fluor® 647 |
| 53-7129 | Anti-Human IL-12/IL-23 p40/p70 Alexa Fluor® 488 |
| 14-7129 | Anti-Human IL-12/IL-23 p40/p70 Purified |
| 11-7139 | Anti-Human IL-13 FITC |
| 11-7179 | Anti-Human IL-17A FITC |
| 12-7179 | Anti-Human IL-17A PE |
| 17-7179 | Anti-Human IL-17A APC |
| 45-7179 | Anti-Human IL-17A PerCP-Cy5.5 |
| 51-7179 | Anti-Human IL-17A Alexa Fluor® 647 |
| 53-7179 | Anti-Human IL-17A Alexa Fluor® 488 |
| 12-7178 | Anti-Human IL-17A PE |
| 51-7178 | Anti-Human IL-17A Alexa Fluor® 647 |
| 17-7222 | Anti-Human/Mouse IL-22 APC |
| 50-7823 | Anti-Human IL-23 p19 eFluor® 660 |
| 50-7023 | Anti-Mouse IL-23 p19 eFluor® 660 |
| 11-7349 | Anti-Human TNF alpha FITC |
| 12-7349 | Anti-Human TNF alpha PE |
| 14-7349 | Anti-Human TNF alpha Purified |
| 17-7349 | Anti-Human TNF alpha APC |
| 25-7349 | Anti-Human TNF alpha PE-Cy7 |
| 45-7349 | Anti-Human TNF alpha PerCP-Cy5.5 |
| 53-7349 | Anti-Human TNF alpha Alexa Fluor® 488 |
| 12-7327 | Anti-Human TNF beta PE |
| 17-7327 | Anti-Human TNF beta APC |
| 11-7319 | Anti-Human IFN gamma FITC |
| 12-7319 | Anti-Human IFN gamma PE |
| 14-7319 | Anti-Human IFN gamma Purified |
| 17-7319 | Anti-Human IFN gamma APC |
| 25-7319 | Anti-Human IFN gamma PE-Cy7 |
| 45-7319 | Anti-Human IFN gamma PerCP-Cy5.5 |
| 51-7319 | Anti-Human IFN gamma Alexa Fluor® 647 |
| 53-7319 | Anti-Human IFN gamma Alexa Fluor® 488 |
| 50-7351 | Anti-Human G-CSF eFluor® 660 |
| 51-7337 | Anti-Human GM-CSF Alexa Fluor® 647 |
| 17-7099 | Anti-Human CCL2 (MCP-1) APC |
| 11-7096 | Anti-CCL2 (MCP-1) FITC |
| 12-7096 | Anti-CCL2 (MCP-1) PE |
| 51-9905 | Anti-Human CCL5 (RANTES) Alexa Fluor® 647 |
| 12-5825 | Anti-Human/Mouse T-bet PE |
| 45-5825 | Anti-Human/Mouse T-bet PerCP-Cy5.5 |

anti-Mouse

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| 11-7011 | Anti-Mouse IL-1 alpha FITC |
| 12-7011 | Anti-Mouse IL-1 alpha PE |
| 11-7021 | Anti-Mouse IL-2 FITC |
| 12-7021 | Anti-Mouse IL-2 PE |
| 16-7021 | Anti-Mouse IL-2 Functional Grade Purified |
| 17-7021 | Anti-Mouse IL-2 APC |
| 25-7021 | Anti-Mouse IL-2 PE-Cy7 |
| 45-7021 | Anti-Mouse IL-2 PerCP-Cy5.5 |
| 53-7021 | Anti-Mouse IL-2 Alexa Fluor® 488 |
| 14-7021 | Anti-Mouse IL-2 Purified |
| 12-7041 | Anti-Mouse IL-4 PE |
| 17-7041 | Anti-Mouse IL-4 APC |
| 53-7041 | Anti-Mouse IL-4 Alexa Fluor® 488 |
| 11-7042 | Anti-Mouse IL-4 FITC |
| 14-7042 | Anti-Mouse IL-4 Purified |
| 25-7042 | Anti-Mouse IL-4 PE-Cy7 |
| 11-7061 | Anti-Mouse IL-6 FITC |
| 12-7061 | Anti-Mouse IL-6 PE |
| 12-7052 | Anti-Human/Mouse IL-5 PE |
| 11-7101 | Anti-Mouse IL-10 FITC |
| 12-7101 | Anti-Mouse IL-10 PE |
| 17-7101 | Anti-Mouse IL-10 APC |
| 45-7101 | Anti-Mouse IL-10 PerCP-Cy5.5 |
| 53-7101 | Anti-Mouse IL-10 Alexa Fluor® 488 |
| 56-7101 | Anti-Mouse IL-10 Alexa Fluor® 700 |
| 16-7123 | Anti-Mouse IL-12/IL-23 p40 Functional Grade Purified |
| 12-7123 | Anti-Mouse IL-12/IL-23 p40 PE |
| 45-7123 | Anti-Mouse IL-12/IL-23 p40 PerCP-Cy5.5 |
| 51-7123 | Anti-Mouse IL-12/IL-23 p40 Alexa Fluor® 647 |
| 12-7133 | Anti-Mouse IL-13 PE |
| 51-7133 | Anti-Mouse IL-13 Alexa Fluor® 647 |
| 53-7172 | Anti-Mouse IL-17A Alexa Fluor® 488 |
| 12-7471 | Anti-Mouse IL-17F PE |
| 53-7471 | Anti-Mouse IL-17F Alexa Fluor® 488 |
| 12-7211 | Anti-Mouse IL-21 PE |
| 14-7321 | Anti-Mouse TNF alpha Purified |
| 46-7321 | Anti-Mouse TNF alpha PerCP-eFluor® 710 |
| 48-7321 | Anti-Mouse TNF alpha eFluor® 450 |
| 11-7321 | Anti-Mouse TNF alpha FITC |
| 12-7321 | Anti-Mouse TNF alpha PE |
| 17-7321 | Anti-Mouse TNF alpha APC |
| 19-7321 | Anti-Mouse TNF alpha Cy5 |
| 53-7321 | Anti-Mouse TNF alpha Alexa Fluor® 488 |
| 11-7423 | Anti-Mouse/Rat TNF alpha FITC |
| 12-7423 | Anti-Mouse/Rat TNF alpha PE |
| 48-7311 | Anti-Mouse IFN gamma eFluor® 450 |
| 11-7311 | Anti-Mouse IFN gamma FITC |
| 12-7311 | Anti-Mouse IFN gamma PE |
| 17-7311 | Anti-Mouse IFN gamma APC |
| 19-7311 | Anti-Mouse IFN gamma Cy5 |
| 25-7311 | Anti-Mouse IFN gamma PE-Cy7 |
| 45-7311 | Anti-Mouse IFN gamma PerCP-Cy5.5 |
| 51-7311 | Anti-Mouse IFN gamma Alexa Fluor® 647 |
| 53-7311 | Anti-Mouse IFN gamma Alexa Fluor® 488 |
| 11-7331 | Anti-Mouse GM-CSF FITC |
| 12-7331 | Anti-Mouse GM-CSF PE |
| 11-7096 | Anti-CCL2 (MCP-1) FITC |
| 12-7096 | Anti-CCL2 (MCP-1) PE |
| 12-5825 | Anti-Human/Mouse T-bet PE |
| 45-5825 | Anti-Human/Mouse T-bet PerCP-Cy5.5 |