

Stimulation Guide for Intracellular Staining of Cytokines/Chemokines

Cytokine/ Chemokine	Clone(s)	Target Cells	Stimulation	Stimulation period	Protein transport blocker	Surface marker
MOUSE						
IL-1 α	ALF-161	Thioglycollate-elicited peritoneal macrophage	LPS (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD107b (MAC-3)
IL-2	JES6-5H4	Splenocytes	PMA (20 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD3
IL-3	MP2-8F8	Purified CD4 ⁺ T cells stimulated with immobilized anti-CD3 (10 μ g/ml) + soluble anti-CD28 (2 μ g/ml) + IL-2 (20 ng/ml) + IL-4 (50 ng/ml) for 2 days, wash and then culture in IL-2 + IL-4 for 3-4 days	PMA (20 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD4*
IL-4	11B11	Purified CD4 ⁺ T cells stimulated with immobilized anti-CD3 (10 μ g/ml) + soluble anti-CD28 (2 μ g/ml) + IL-2 (20 ng/ml) + IL-4 (50 ng/ml) for 2 days, wash and then culture in IL-2 + IL-4 for 3-4 day	PMA (20 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD4*
IL-5	TRFK	Purified CD4 ⁺ T cells stimulated with immobilized anti-CD3 (10 μ g/ml) + soluble anti-CD28 (2 μ g/ml) + IL-2 (20 ng/ml) + IL-4 (50 ng/ml) for 2 days, wash and then culture in IL-2 + IL-4 for 3-4 day	PMA (20 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD4*
IL-6	MP5-20F3	Thioglycollate-elicited peritoneal macrophage	LPS (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD107b (MAC-3)
IL-9	RM9A4	Purified CD4 ⁺ T cells stimulated with immobilized anti-CD3 (5 μ g/ml) + soluble anti-CD28 (5 μ g/ml) + IL-2 (20 ng/ml) + IL-4 (10 ng/ml) + TGF- β 1 (10 ng/mL) + anti-mouse IFN- γ (10 μ g/mL) for 3 days	PMA (500 ng/ml) plus ionomycin (500 ng/ml)	4-6 hours	Brefeldin A	CD4*
IL-10	JES5-16E3	Purified CD4 ⁺ T cells stimulated with immobilized anti-CD3 (10 μ g/ml) + soluble anti-CD28 (2 μ g/ml) + IL-2 (20 ng/ml) + IL-4 (50 ng/ml) for 2 days, wash and then culture in IL-2 + IL-4 for 3-4 day	PMA (20 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD4*
IL-12	C15.6	Thioglycollate-elicited peritoneal macrophage	IFN- γ (10 ng/ml) prime for 2 hours, then add LPS (1 μ g/ml)	18-24 hours	monensin or brefeldin A	CD107b (MAC-3)

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IL-17A	TC11-18H10.1	Purified CD4+ T cells stimulated with immobilized anti-CD3 (2 µg/ml) + soluble anti-CD28 (5 µg/ml), IL-6 (50 ng/ml), TGF-β1 (1 ng/ml), anti-mouse IL-4 (10 µg/ml), anti-mouse IFN-γ (10 µg/ml), and 300 nM of FICZ cultured for 3 days. Alternatively, mouse IL-23 (5 ng/ml) can be used in place of FICZ.	PMA (500 ng/ml) + ionomycin (500 ng/ml)	4-6 hours	Brefeldin A	CD4*
IL-17F	9D3.1C8	Purified CD4+ T cells stimulated with immobilized anti-CD3 (2 µg/ml) + soluble anti-CD28 (5 µg/ml), IL-6 (50 ng/ml), TGF-β1 (1 ng/ml), anti-mouse IL-4 (10 µg/ml), anti-mouse IFN-γ (10 µg/ml), and 300 nM of FICZ cultured for 3 days. Alternatively, mouse IL-23 (5 ng/ml) can be used in place of FICZ.	PMA (500 ng/ml) + ionomycin (500 ng/ml)	4-6 hours	Brefeldin A	CD4*
IL-21	BL25168	Purified CD4+ T cells stimulated with immobilized anti-CD3 (2 µg/ml) + soluble anti-CD28 (5 µg/ml), IL-6 (50 ng/ml), TGF-β1 (1 ng/ml), anti-mouse IL-4 (10 µg/ml), anti-mouse IFN-γ (10 µg/ml), and 300 nM of FICZ cultured for 3 days. Alternatively, mouse IL-23 (5 ng/ml) can be used in place of FICZ.	PMA (500 ng/ml) + ionomycin (500 ng/ml)	4-6 hours	Brefeldin A	CD4*
IL-22	Poly5164	Purified CD4+ T cells stimulated with immobilized anti-CD3 (2 µg/ml) + soluble anti-CD28 (5 µg/ml), IL-6 (50 ng/ml), TGF-β1 (1 ng/ml), anti-mouse IL-4 (10 µg/ml), anti-mouse IFN-γ (10 µg/ml), and 300 nM of FICZ cultured for 3 days. Alternatively, mouse IL-23 (5 ng/ml) can be used in place of FICZ.	PMA (500 ng/ml) + ionomycin (500 ng/ml)	4-6 hours	Brefeldin A	CD4*
IL-27	MM27-7B1	Thioglycolate-elicited Balb/c peritoneal macrophages primed with IFN-γ (100 ng/ml) for 2 hours	LPS (1 µg/ml)	4-6 hours	monensin or brefeldin A	CD107b (MAC-3)
GM-CSF	MP1-22E9	Splenocytes stimulated with immobilized anti-CD3 (10 µg/ml) + soluble anti-CD28 (2 µg/ml) for 2 days, wash and then culture in IL-2 (20 ng/ml) + IL-4 (50 ng/ml) for 3-4 day	PMA (20 ng/ml) plus ionomycin (1 µg/ml)	4-6 hours	monensin or brefeldin A	CD3
IFN-γ	XMG1.2	Splenocytes	PMA (20 ng/ml) plus ionomycin (1 µg/ml)	4-6 hours	monensin or brefeldin A	CD3

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MCP-1	2H5	Thioglycollate-elicited peritoneal macrophage	LPS (1 µg/ml)	4-6 hours	monensin or brefeldin A	CD107b (MAC-3)
TNF-α	MP6-XT22	Splenocytes	PMA (20 ng/ml) plus ionomycin (1 µg/ml)	4-6 hours	monensin or brefeldin A	CD3
HUMAN						
IL-1α	364-3B3-14	PBMCs	LPS (100 ng/ml)	4-6 hours	monensin or brefeldin A	CD14*
IL-1β	JK1B-1	PBMCs	LPS (100 ng/ml)	4-6 hours	monensin or brefeldin A	CD14*
IL-2	MQ1-17H12	PBMCs	PMA (50 ng/ml) plus ionomycin (1 µg/ml)	4-6 hours	monensin or brefeldin A	CD3
IL-3	BVD3-1F9	Purified CD4+ T cells stimulated with immobilized anti-CD3 (10 µg/ml) + soluble anti-CD28 (5 µg/ml) + IL-2 (20 ng/ml) + IL-4 (50 ng/ml) for 2 days, wash and then culture in IL-2 + IL-4 for 3-4 days	PMA (50 ng/ml) plus ionomycin (1 µg/ml)	4-6 hours	monensin or brefeldin A	CD4*
IL-4	MP4-25D2 or 8D4-8	PBMCs	PMA (50 ng/ml) plus ionomycin (1 µg/ml)	4-6 hours	monensin or brefeldin A	CD4*
IL-5	JES1-39D10	Purified CD4+ T cells stimulated with immobilized anti-CD3 (10 µg/ml) + soluble anti-CD28 (5 µg/ml) + IL-2 (20 ng/ml) + IL-4 (50 ng/ml) for 2 days, wash and then culture in IL-2 + IL-4 for 3-4 days	PMA (50 ng/ml) plus ionomycin (1 µg/ml)	4-6 hours	monensin or brefeldin A	CD4*
IL-6	MQ2-13A5	PBMCs	LPS (100 ng/ml)	4-6 hours	monensin or brefeldin A	CD14*
IL-8	JK8-1	PBMCs	LPS (100 ng/ml)	4-6 hours	monensin or brefeldin A	CD14*
IL-9	MH9A4	Purified CD4+ T cells stimulated with immobilized anti-CD3 (10 µg/ml) + soluble anti-CD28 (5 µg/ml) + IL-2 (20 ng/ml) + IL-4 (50 ng/ml) for 2 days, wash and then culture in IL-2 + IL-4 for 3-4 days	PMA (50 ng/ml) plus ionomycin (1 µg/ml)	4-6 hours	monensin or brefeldin A	CD4*
IL-10	JES3-9D7 or JES3-19F1	PBMCs	LPS (100 ng/ml)	18-24 hours	monensin or brefeldin A	CD14*

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IL-12 p40	C11.5	PBMCs	IFN- γ (100 ng/ml) prime for 2 hours, then add LPS (1 μ g/ml)	18-24 hours	monensin or brefeldin A	CD14*
IL-13	JES10-5A2	Purified CD4 ⁺ T cells stimulated with immobilized anti-CD3 (10 μ g/ml) + soluble anti-CD28 (5 μ g/ml) + IL-2 (20 ng/ml) + IL-4 (50 ng/ml) for 2 days, wash and then culture in IL-2 + IL-4 for 3-4 days	PMA (50 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD4*
IL-17A	BL168	PBMCs	PMA (50 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD4*
IL-21	3A3-N2 or 4BG1	PBMCS	PMA (50 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD4*
IL-22	BG/IL22	PBMCs	PMA (50 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD4*
GM-CSF	BVD2-21C11	PBMCs stimulated with soluble anti-CD3 (UCHT1, 1 μ g/ml) + soluble anti-CD28 (5 μ g/ml) for 2 days, wash and then culture in IL-2 (20 ng/ml) + IL-4 (50 ng/ml) for 3-4 days	PMA (50 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD3
IFN- γ	4S.B3 or B27	PBMCs	PMA (50 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD3
MCP-1	5D3-F7 or 2H5	PBMCs	LPS (100 ng/ml)	18-24 hours	monensin or brefeldin A	CD14*
TNF- α	MAB11	PBMCs	PMA (50 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD3
TNF- β	359-81-11	PBMCs stimulated with soluble anti-CD3 (UCHT1, 1 μ g/ml) + soluble anti-CD28 (5 μ g/ml) for 2 days, wash and then culture in IL-2 (20 ng/ml) + IL-4 (50 ng/ml) for 3-4 days	PMA (50 ng/ml) plus ionomycin (1 μ g/ml)	4-6 hours	monensin or brefeldin A	CD3

Notes: *Surface expression of CD4 is down-modulated by PMA/Ionomycin stimulation, and surface expression of CD14 is down-regulated by LPS stimulation.