

Materials List for:

Phospho Flow Cytometry with Fluorescent Cell Barcoding for Single Cell Signaling Analysis and Biomarker Discovery

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URL: <https://www.jove.com/video/58386>

DOI: [doi:10.3791/58386](https://doi.org/10.3791/58386)

Materials

Name	Company	Catalog Number	Comments
RPMI 1640 GlutaMAX	ThermoFisher Scientific	61870-010	Cell culture medium
Fetal bovine serum	ThermoFisher Scientific	10270169	Additive to cell culture medium
Sodium pyruvate	ThermoFisher Scientific	11360-039	Additive to cell culture medium
MEM non-essential amino acids	ThermoFisher Scientific	11140-035	Additive to cell culture medium
Lymphoprep	Alere Technologies AS	1114547	Density gradient medium
Anti-IgM	Southern Biotech	2022-01	For stimulation of the B cell receptor
BD Phosflow Fix Buffer I	BD	557870	Fixation buffer
BD Phosflow Perm Buffer III	BD	558050	Permeabilization buffer
Alexa Fluor 488 5-TFP	ThermoFisher Scientific	A30005	Barcoding reagent
Pacific Blue Succinimidyl Ester	ThermoFisher Scientific	P10163	Barcoding reagent
Pacific Orange Succinimidyl Ester, Triethylammonium Salt	ThermoFisher Scientific	P30253	Barcoding reagent
Compensation beads	<i>Defined by user</i>		Correct species reactivity
Falcon tubes	<i>Defined by user</i>		
Eppendorf tubes	<i>Defined by user</i>		
96 well V-bottom plates	<i>Defined by user</i>		Compatible with the flow cytometer
Centrifuges	<i>Defined by user</i>		For Eppendorf tubes, Falcon tubes and plates
Water bath	<i>Defined by user</i>		Temperature regulated
Flow cytometer	<i>Defined by user</i>		With High Throughput Sampler (HTS)
Name	Company	Catalog Number	Comments
Antigen			
AKT (pS473)	Cell Signaling Technologies	4075	Clone: D9E Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., <i>Oncotarget</i> , 9(10):9273-9284 Parente-Ribes et al., 2016, Spleen tyrosine kinase inhibitors reduce..., <i>Haematologica</i> , 101(2):e59-62 Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., <i>Biochem J</i> , 460(3):399-410 Kalland et al., 2012, Modulation of proximal signaling in normal and transformed..., <i>Exp Cell Res</i> , 318(14):1611-9

ATF-2 (pT71)	Santa Cruz Biotechnology	sc-8398	Clone: F-1 Reference: Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., Biochem J, 460(3):399-410 Pollheimer et al., 2013, Interleukin-33 drives a proinflammatory endothelial..., Arterioscler Thromb Vasc Biol, 33(2):e47-55
BLNK (pY84)	Beckton Dickinson Pharmingen	558443	Clone: J117-1278 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Parente-Ribes et al., 2016, Spleen tyrosine kinase inhibitors reduce..., Haematologica, 101(2):e59-62 Kalland et al., 2012, Modulation of proximal signaling in normal and transformed..., Exp Cell Res, 318(14):1611-9 Myklebust et al., 2017, Distinct patterns of B-cell receptor signaling in..., Blood, 129(6): 759-770
Btk (pY223)/Itk (pY180)	Beckton Dickinson Pharmingen	564846	Clone: N35-86 Reference: Myklebust et al., 2017, Distinct patterns of B-cell receptor signaling in..., Blood, 129(6): 759-770
Btk (pY551)	Beckton Dickinson Pharmingen	558129	Clone: 24a/BTK (Y551) Reference: Kalland et al., 2012, Modulation of proximal signaling in normal and transformed..., Exp Cell Res, 318(14):1611-9
Btk (pY551)/Itk (pY511)	Beckton Dickinson Pharmingen	558134	Clone: 24a/BTK (Y551) Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Parente-Ribes et al., 2016, Spleen tyrosine kinase inhibitors reduce..., Haematologica, 101(2):e59-62
CD3 ζ (pY142)	Beckton Dickinson Pharmingen	558489	Clone: K25-407.69 Reference: Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., Biochem J, 460(3):399-410
Histone H3 (pS10)	Cell Signaling Technologies	9716	Clone: D2C8 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284
I κ B α	Cell Signaling Technologies	5743	Clone: L35A5 Reference: Myklebust et al., 2017, Distinct patterns of B-cell receptor signaling in..., Blood, 129(6): 759-770
LAT (pY171)	Beckton Dickinson Pharmingen	558518	Clone: I58-1169 Reference: Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., Biochem J, 460(3):399-410
Lck (pY505)	Beckton Dickinson Pharmingen	558577	Clone: 4/LCK-Y505

			Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284
MEK1 (pS298)	Beckton Dickinson Pharmingen	560043	Clone: J114-64 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., Biochem J, 460(3):399-410
NF-kB p65 (pS529)	Beckton Dickinson Pharmingen	558422	Clone: K10-895.12.50 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., Biochem J, 460(3):399-410 Kalland et al., 2012, Modulation of proximal signaling in normal and transformed..., Exp Cell Res, 318(14):1611-9 Pollheimer et al., 2013, Interleukin-33 drives a proinflammatory endothelial..., Arterioscler Thromb Vasc Biol, 33(2):e47-55
NF-kB p65 (pS536)	Cell Signaling Technologies	4887	Clone: 93H1 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., Biochem J, 460(3):399-410 Kalland et al., 2012, Modulation of proximal signaling in normal and transformed..., Exp Cell Res, 318(14):1611-9
p38 MAPK (pT180/Y182)	Cell Signaling Technologies	4552	Clone: 28B10 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., Biochem J, 460(3):399-410 Pollheimer et al., 2013, Interleukin-33 drives a proinflammatory endothelial..., Arterioscler Thromb Vasc Biol, 33(2):e47-55
p44/42 MAPK (pT202/Y204)	Cell Signaling Technologies	4375	Clone: E10 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Parente-Ribes et al., 2016, Spleen tyrosine kinase inhibitors reduce..., Haematologica, 101(2):e59-62

			Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., Biochem J, 460(3):399-410 Kalland et al., 2012, Modulation of proximal signaling in normal and transformed..., Exp Cell Res, 318(14):1611-9 Pollheimer et al., 2013, Interleukin-33 drives a proinflammatory endothelial..., Arterioscler Thromb Vasc Biol, 33(2):e47-55
p53 (pS15)	Cell Signaling Technologies	NN	Clone: 16G8 Reference: Irish et al., 2007, Flt3 Y591 duplication and Bcl-2 overexpression..., Blood, 109(6):2589-96
p53 (pS20)	Cell Signaling Technologies	NN	Clone: Polyclonal Reference: Irish et al., 2007, Flt3 Y591 duplication and Bcl-2 overexpression..., Blood, 109(6):2589-96
p53 (pS37)	Cell Signaling Technologies	NN	Clone: Polyclonal Reference: Irish et al., 2007, Flt3 Y591 duplication and Bcl-2 overexpression..., Blood, 109(6):2589-96
p53 (pS46)	Cell Signaling Technologies	NN	Clone: Polyclonal Reference: Irish et al., 2007, Flt3 Y591 duplication and Bcl-2 overexpression..., Blood, 109(6):2589-96
p53 (pS392)	Cell Signaling Technologies	NN	Clone: Polyclonal Reference: Irish et al., 2007, Flt3 Y591 duplication and Bcl-2 overexpression..., Blood, 109(6):2589-96
PLCγ2 (pY759)	Beckton Dickinson Pharmingen	558498	Clone: K86-689.37 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Myklebust et al., 2017, Distinct patterns of B-cell receptor signaling in..., Blood, 129(6): 759-770
Rb (pS807/pS811)	Beckton Dickinson Pharmingen	558590	Clone: J112-906 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Pollheimer et al., 2013, Interleukin-33 drives a proinflammatory endothelial..., Arterioscler Thromb Vasc Biol, 33(2):e47-55
S6-Ribos. Prot. (pS235/236)	Cell Signaling Technologies	4851	Clone: D57.2.2E Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284
SAPK/JNK (pT183/Y185)	Cell Signaling Technologies	9257	Clone: G9 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-

			protein levels in..., Oncotarget, 9(10):9273-9284 Pollheimer et al., 2013, Interleukin-33 drives a proinflammatory endothelial..., Arterioscler Thromb Vasc Biol, 33(2):e47-55
SLP76 (pY128)	Beckton Dickinson Pharmingen	558438	Clone: J141-668.36.58 Reference: Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., Biochem J, 460(3):399-410
STAT1 (pY701)	Beckton Dickinson Pharmingen	612597	Clone: 4a Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Myklebust et al., 2017, Distinct patterns of B-cell receptor signaling in..., Blood, 129(6): 759-770
STAT3 (pY705)	Beckton Dickinson Pharmingen	557815	Clone: 4/P-STAT3 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284
STAT4 (pY693)	Zymed/ThermoFisher Scientific	71-7900	Clone: Polyclonal Reference: Uzel et al., 2001, Detection of intracellular phosphorylated STAT-4 by flow cytometry, Clin Immunol, 100(3): 270-6
STAT5 (pY694)	Beckton Dickinson Pharmingen	612599	Clone: 47/Stat5(pY694) Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., Biochem J, 460(3):399-410 Myklebust et al., 2017, Distinct patterns of B-cell receptor signaling in..., Blood, 129(6): 759-770
STAT6 (pY641)	Beckton Dickinson Pharmingen	612601	Clone: 18/P-Stat6 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284
SYK (pY525/Y526)	Cell Signaling Technologies	12081	Clone: C87C1 Reference: Myhrvold et al., 2018, Single cell profiling of phospho-protein levels in..., Oncotarget, 9(10):9273-9284 Parente-Ribes et al., 2016, Spleen tyrosine kinase inhibitors reduce..., Haematologica, 101(2):e59-62
ZAP70/SYK (pY319/Y352)	Beckton Dickinson Pharmingen	557817	Clone: 17A/P-ZAP70 Reference: Skånland et al., 2014, T-cell co-stimulation through the CD2 and CD28..., Biochem J, 460(3):399-410 Kalland et al., 2012, Modulation of proximal signaling in normal and transformed..., Exp Cell Res, 318(14):1611-9

			Myklebust et al., 2017, Distinct patterns of B-cell receptor signaling in..., Blood, 129(6): 759-770
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