

Materials List for:

# A Chromatin Immunoprecipitation Assay to Identify Novel NFAT2 Target Genes in Chronic Lymphocytic Leukemia

Alexander R. Fuchs<sup>1</sup>, Melanie Märklin<sup>1</sup>, Jonas S. Heitmann<sup>1</sup>, Stefan Futterknecht<sup>1</sup>, Michael Haap<sup>2</sup>, Stefan Wirths<sup>1</sup>, Hans-Georg Kopp<sup>1</sup>, Clemens Hinterleitner<sup>1</sup>, Daniela Dörfel<sup>1</sup>, Martin R. Müller<sup>1</sup>

<sup>1</sup>Dept. of Hematology, Oncology and Immunology, University of Tübingen

<sup>2</sup>Dept. of Endocrinology, Diabetology, Clinical Pathology and Metabolism, University of Tübingen

Correspondence to: Martin R. Müller at [martin.mueller@med.uni-tuebingen.de](mailto:martin.mueller@med.uni-tuebingen.de)

URL: <https://www.jove.com/video/58270>

DOI: [doi:10.3791/58270](https://doi.org/10.3791/58270)

## Materials

Name	Company	Catalog Number	Comments
1 X PBS	Sigma Aldrich	D8537	
1.5 mL tube shaker Themomixer comfort	Eppendorf	5355 000.011	Can be substituted with similar instruments
10X Bolt Sample Reducing Agent	Thermo Scientific	B0009	
20X Bolt MES SDS Running Buffer	Thermo Scientific	B0002	
37 % Formaldehyde p.a., ACS	Roth	4979.1	
4X Bolt LDS Sample Buffer	Thermo Scientific	B0007	
Anti-NFAT2 antibody	Alexis	1008505	Clone 7A6
Anti-NFAT2 antibody	Cell Signaling	8032S	Clone D15F1
Anti-NFAT2 antibody ChIP Grade	Abcam	ab2796	Clone 7A6
big Centrifuge	Eppendorf	5804R	Can be substituted with similar instruments
CD19-FITC mouse Anti-human	BD Biosciences	555412	Clone HIB19
CD5-PE mouse Anti-human CD5	BD Biosciences	555353	Clone UCHT2
Density gradient medium Biocoll (Density 1,077 g/ml)	Merck	L 6115	
DNA LoBind Tube 1.5 mL	eppendorf	22431021	
FBS superior	Merck	S0615	
Flow Cytometer	BD Biosciences	FACSCalibur	Can be substituted with similar instruments
Halt Protease and Phosphatase Inhibitor Cocktail (100X)	Thermo Scientific	78440	
iBlot 2 Gel Transfer Device	Thermo Scientific	IB21001	
iBlot 2 Transfer Stacks, nitrocellulose, regular size	Thermo Scientific	IB23001	
iDeal ChIP-seq kit for Histones	Diagenode	C01010059	
Ionomycin calcium salt	Sigma Aldrich	I3909	
IRDye 680LT Donkey anti-Rabbit IgG (H + L), 0.5 mg	LI-COR Biosciences	926-68023	
IRDye 800CW Goat anti-Mouse IgG (H + L), 0.1 mg	LI-COR Biosciences	925-32210	
LI-COR Odyssey Infrared Imaging System	LI-COR Biosciences	B446	
LightCycler 480 Multiwell Plate 96, white	Roche	4729692001	Can be substituted with other plates in different real-time PCR instruments

Lysing Solution OptiLyse B	Beckman Coulter	IM1400	
M220 AFA-grade water	Covaris	520101	
M220 Focused-ultrasonicator	Covaris	500295	
Magnetic rack, DynaMag-15 Magnet	Thermo Scientific	12301D	Can be substituted with similar instruments
MEM Non-Essential Amino Acids Solution 100X	Thermo Scientific	11140050	
Microscope Axiovert 25	Zeiss	451200	Can be substituted with similar instruments
microTUBE AFA Fiber Pre-Slit Snap-Cap 6x16mm	Covaris	520045	
Neubauer improved counting chamber	Karl Hecht GmbH & Co KG	40442012	Can be substituted with similar instruments
NH4 Heparin Monovette	Sarstedt	02.1064	
Nuclease-free water	Promega	P1193	
NuPAGE 4-12% Bis-Tris Protein Gels, 1.0 mm, 15-well	Thermo Scientific	NP0323BOX	
Odyssey® Blocking Buffer (TBS) 500 mL	LI-COR Biosciences	927-50000	
Penicillin/Streptomycin 100X	Merck	A2213	
PerfeCTa SYBR Green FastMix	Quanta Bio	95072-012	
PMA	Sigma Aldrich	P1585	
Primer <i>CD40L</i> promotor region forward	Sigma Aldrich		5'-ACTCGGTGTTAGCCAGG-3'
Primer <i>CD40L</i> promotor region reverse	Sigma Aldrich		5'-GGGCTCTGGGTGCTATTGT-3'
Primer <i>IL-2</i> promotor region forward	Sigma Aldrich		5'-TCCAAAGAGTCATCAGAAGAG-3'
Primer <i>IL-2</i> promotor region reverse	Sigma Aldrich		5'-GGCAGGAGTTGAGGTTACTGT-3'
Primer <i>LCK</i> promotor region forward	Sigma Aldrich		5'-CAGGCAAAACAGGCACACAT-3'
Primer <i>LCK</i> promotor region reverse	Sigma Aldrich		5'-CCTCCAGTGA CTCTGTTGGC-3'
Rabbit mAb IgG XP Isotype Control	Cell Signaling	# 3900S	Clone DA1E
Real-time PCR instrument	Roche	LightCycler 480	Can be substituted with similar instruments
Roller mixers	Phoenix Instrument	RS-TR 5	
RPMI 1640 Medium, GlutaMAX Supplement	Thermo Scientific	61870010	
Safety-Multifly-needle 21G	Sarstedt	851638235	
SeeBlue Plus2 Pre-stained Protein Standard	Thermo Scientific	LC5925	
Shaker Duomax 1030	Heidolph Instruments	543-32205-00	Can be substituted with similar instruments
small Centrifuge	Thermo Scientific	Heraeus Fresco 17	Can be substituted with similar instruments
Sodium Pyruvate	Thermo Scientific	11360070	
β-Mercaptoethanol	Thermo Scientific	21985023	
Tris Buffered Saline (TBS-10X)	Cell Signaling	#12498	
Trypan Blue solution	Sigma Aldrich	93595-50ML	