

Materials List for:

# Quantitative Immunoblotting of Cell Lines As a Standard to Validate Immunofluorescence for Quantifying Biomarker Proteins in Routine Tissue Samples

Alison M. Moore<sup>1,2</sup>, Lee R. Boudreau<sup>3</sup>, Shakeel Virk<sup>3</sup>, David P. LeBrun<sup>1,2</sup>

<sup>1</sup>Department of Pathology and Molecular Medicine, Queen's University

<sup>2</sup>Division of Cancer Biology and Genetics, Queen's Cancer Research Institute

<sup>3</sup>Queen's Laboratory for Molecular Pathology, Department of Pathology and Molecular Medicine, Queen's University

Correspondence to: David P. LeBrun at [dpl1@queensu.ca](mailto:dpl1@queensu.ca)

URL: <https://www.jove.com/video/58735>

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## Materials

Name	Company	Catalog Number	Comments
697	DSMZ	ACC 42	Cell line
JeKo-1	ATCC	CRL-3006	Cell line
Jurkat	ATCC	TIB-152	Cell line
RCH-ACV	DSMZ	ACC 548	Cell line
Granta-519	DSMZ	ACC 342	Cell line
REH	DSMZ	ACC 22	Cell line
Raji	ATCC	CCL-86	Cell line
HeLa	ATCC	CCL-2	Cell line
Trypsin/EDTA solution	Invitrogen	R001100	For detaching adhesive cells
Fetal bovine serum (FBS)	Wisent Inc.	81150	To neutralize trypsin
Neutral Buffered Formalin	Protocol	245-684	For fixing cell pellets
UltraPure low melting point agarose	Invitrogen	15517-022	For casting cell pellets
Mouse monoclonal anti-human Bcl-2 antibody, clone 124	Dako (Agilent)	cat#: M088729-2, RRID: 2064429	To detect Bcl-2 by immunofluorescence and immunoblot (lot#: 00095786)
Ventana Discovery XT	Roche	-	For automation of immunofluorescence staining
EnVision+ System- HRP labelled polymer (anti-mouse)	Dako (Agilent)	K4000	For immunofluorescence signal amplification
EnVision+ System- HRP labelled polymer (anti-rabbit)	Dako (Agilent)	K4002	For immunofluorescence signal amplification
Cyanine 5 tyramide reagent	Perkin Elmer	NEL745001KT	For immunofluorescence signal amplification
Aperio ImageScope	Leica Biosystems	-	To view scanned slides
HALO image analysis software	Indica Labs	-	For quantification of immunofluorescence
Protease inhibitors (Halt protease inhibitor cocktail, 100X)	Thermo Scientific	1862209	To add to RIPA buffer
Ethylenediaminetetraacetic acid (EDTA)	BioShop	EDT001	For RIPA buffer
NP-40	BDH Limited	56009	For RIPA buffer
Sodium deoxycholate	Sigma-Aldrich	D6750	For RIPA buffer
Glycerol	FisherBiotech	BP229	For Laemmli buffer

Bromophenol blue	BioShop	BRO777	For Laemlli buffer
Dithiothreitol (DTT)	Bio-Rad	161-0611	For Laemlli buffer
Bovine serum albumin (BSA)	BioShop	ALB001	For immunoblot washes
Protein ladder (Precision Plus Protein Dual Color Standards)	Bio-Rad	161-0374	For running protein gel
Filter paper (Extra thick blot paper)	Bio-Rad	1703969	For blotting transfer
Nitrocellulose membrane	Bio-Rad	162-0115	For blotting transfer
Trans-blot SD semi-dry transfer cell	Bio-Rad	1703940	For semi-dry transfer
Skim milk powder (Nonfat dry milk)	Cell Signaling Technology	9999S	For blocking buffer
Tween 20	BioShop	TWN510	For wash buffer
GAPDH rabbit monoclonal antibody	Epitomics	2251-1	Primary antibody of control protein (lot#: YE101901C)
Goat anti-mouse IgG (HRP conjugated) antibody	abcam	cat#: ab6789, RRID: AB_955439	Secondary antibody for immunoblot
Goat anti-rabbit IgG (HRP conjugated) antibody	abcam	cat#: ab6721, RRID: AB_955447	Secondary antibody for immunoblot (lot#: GR3192725-5)
Clarity Western ECL substrates	Bio-Rad	1705060	For immunoblot signal detection
Amersham Imager 600	GE Healthcare Life Sciences	29083461	For immunoblot signal detection
ImageJ software	Freeware, NIH	-	For densitometry analysis