

Materials List for:

Workflow for High-content, Individual Cell Quantification of Fluorescent Markers from Universal Microscope Data, Supported by Open Source Software

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Materials

Name	Company	Catalog Number	Comments
AllStars negative control siRNA	Qiagen	1027280	Negative control siRNA.
CDK6 siRNA	Dharmacon/ Custom synthesis	NA	Antisense sequence: 5' CUCUAGGCCAGUCUUCUUCUU
RB1 siRNA	Dharmacon/ Custom synthesis	NA	Antisense sequence: 5' GGUUCAACUACGCGUGUAATT
5x siRNA buffer	Thermo Scientific	B-002000-UB-100	To be diluted with nuclease-free, non-DEPC treated water.
Nulcease-free water (non-DEPC)	Applied Biosystems	AM9937	For dilution of siRNA buffer.
Hiperfect	Qiagen	301705	Transfection lipid.
DMEM	Life Technologies	41966052	Pyruvate and high-glucose supplemented tissue culture media.
96 well tissue culture plate	Falcon	3072	Plain, 96 well tissue culture plate for the parallel, compartmentalized mixing of transfection complexes.
Packard Viewplate	Perkin Elmer LAS	6005182	96 well TC plate with optical base on which cells are transfected, grown, fixed and eventually imaged. Supplied with opaque, adhesive plate seals, which are used during storage of used plates.
Breathable membrane	Alpha Labs	LW2783	Sterile, gas-permeable, adhesive membrane.
Neutral buffered formalin, 10%	Sigma-Aldrich	HT5012-1CS	10% Formalin (4% formaldehyde) fixative used neat in protocol.
Triton X-100	Sigma-Aldrich	X100PC	Non-ionic detergent

Name	Company	Catalog Number	Comments
Tris	Sigma-Aldrich	T1503	Trizma base (tris(hydroxymethyl)aminomethane)
Tween 20	Sigma-Aldrich	P2287	For plate wash solution.
Anti-P-S780 RB1 antibody	Abcam	ab32513	Rabbit monoclonal
AlexaFluor647 Anti-rabbit	Invitrogen	A21245	Highly cross-adsorbed, fluorescently labelled secondary antibody.
Hoechst 33342 (Bisbenzimidazole)	Sigma-Aldrich	B2261	Fluorescent, chromatin-intercalating, DNA dye.
Permeabilization solution	NA	NA	0.1% Triton X-100 in 50 mM Tris-buffered saline, pH 8.0.

Plate wash solution	NA	NA	Tris-buffered saline containing 0.1% Tween-20.
Block solution	NA	NA	5% powdered milk in Tris-buffered saline and 0.1% Tween-20. For blocking plate prior to immunostaining and dilution of antibodies.
Cell Profiler 2.1	Broad Institute		http://www.cellprofiler.org/download.shtml
Active Perl Community Edition	ActiveState		http://www.activestate.com/activeperl/downloads
R programming environment	The R Foundation		http://www.r-project.org
Rstudio	Rstudio		http://www.rstudio.com/