

Materials List for:

A Rhodopsin Transport Assay by High-Content Imaging Analysis

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URL: <https://www.jove.com/video/58703>

DOI: [doi:10.3791/58703](https://doi.org/10.3791/58703)

Materials

Name	Company	Catalog Number	Comments
U2OS (rhodopsin-Venus) cells	NA	NA	Stable cells generated from U2OS cells
U2OS (T4R-rhodopsin-Venus) cells	NA	NA	Stable cells generated from U2OS cells
U2OS (P23H-rhodopsin-Venus) cells	NA	NA	Stable cells generated from U2OS cells
U2OS (P53R-rhodopsin-Venus) cells	NA	NA	Stable cells generated from U2OS cells
U2OS (C110Y-rhodopsin-Venus) cells	NA	NA	Stable cells generated from U2OS cells
U2OS (D190N-rhodopsin-Venus) cells	NA	NA	Stable cells generated from U2OS cells
U2OS (P267L-rhodopsin-Venus) cells	NA	NA	Stable cells generated from U2OS cells
DMEM high glucose	Genesee Scientific	25-500	With L-Glutamine, sodium pyruvate
Fetal bovine serum (FBS)	Gibco	16140071	Heat inactivated
Plasmocin	InvivoGen	ant-mpt	Mycoplasma elimination reagent
Penicillin-Streptomycin (100X)	Gibco	15140122	100X concentrated antibiotic solutions to prevent bacteria contamination of cell cultures
Trypsin-EDTA	Genesee Scientific	25-510	0.25%, 1mM EDTA in HBSS without calcium and magnesium
Poly-L-lysine solution	Sigma-Aldrich	P4707-50ML	Mol wt 70,000-150,000, 0.01%, sterile-filtered, BioReagent, suitable for cell culture
CellCarrier-384 Ultra Microplates	PerkinElmer	6057300	384-well tissue culture-treated microplates with black well walls and an optically -clear cyclic olefin bottom for imaging cells in high content analysis
Sterile 96-well plate	Eppendorf	30730119	Tissue culture treated with lid flat bottom, sterile, free of detectable pyrogens, RNase, DNase and DNA. Non-cytotoxic
Phosphate Buffered Saline (PBS)	Invitrogen	AM9625	10 x PBS Buffer, pH 7.4
DMSO	Sigma-Aldrich	D4540	>99.5%, cell culture tested
9- <i>cis</i> -retinal	Sigma-Aldrich	R5754	
Compounds tested	Selleckchem/Life Chemicals/ Custom synthesized	NA	Compounds were purchased from different vendors or custom synthesized
B6-30 anti-rhodopsin antibody	Novus	NBP2-25160	Gift from Dr. Krzysztof Palczewski

Cy3-conjugated goat anti-mouse secondary antibody	Jackson ImmunoResearch Laboratories, Inc	115-165-146	
16% paraformaldehyde	Thermo Fisher Scientific	28908	Methanol-free
10% Normal Goat Serum	Thermo Fisher Scientific	50062Z	Blocking buffer
Hoechst 33342, Trihydroch	Invitrogen	H3570	Nuclear staining solution
High-content imager	Molecular Devices	ImageXpress	ImageXpress® Micro Confocal High-Content Imaging System
MetaXpress high-content image acquisition and analysis software	Molecular Devices	MetaXpress	High-content image acquisition and analysis software
Multichannel pipette (0.5-10 µL)	Rainin	17013802	Manual 8-channel pipette, 0.5-10 µL
Multichannel pipette (0.5-10c	Rainin	17013805	Manual 8-channel pipette, 20-200 µL
Electronic multichannel pipette (10-200 µL)	Thermo Scientific	14-3879-56BT	Electronic multichannel pipette for 96- and 384-well microplate pipetting tasks
50ml Reagent Reservoir	Genesee Scientific	28-125	Reagent reservoir for multichannel pipette dispensing
8-Channel aspirator	ABC Scientific	EV503	8-Channel stainless steel adaptor for aspirating liquids from 96- or 384-well plates
Excel spreadsheet software	Microsoft	Excel2016	The spreadsheet software for data analysis and heatmap generation
Origin2018 scientific data analysis and graphing software	OriginLab	Origin2018	The data analysis software for generating the dose response curves