

# Induction and Analysis of Oxidative Stress in *Sleeping Beauty* Transposon-Transfected Human Retinal Pigment Epithelial Cells

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jove.com/video/61957

## Materials

Name	Company	Catalog Number	Comments
24-well plates	Corning	353047	
6-well plates	Greiner	7657160	
96-well culture plate white with clear flat bottom	Costar	3610	Allows to check the cells before measuring the luminescence (GSH-Glo Assay)
96-well plates	Corning	353072	
Acrylamid 40%	Biorad	161-0144	
Amphotericin B	AMIMED	4-05F00-H	
Antibody anti-GMCSF	ThermoFisher Scientific	PA5-24184	
Antibody anti-mouse IgG/IgA/IgM	Agilent	P0260	
Antibody anti-PEDF	Santa Cruz Biotechnology Inc	sc-390172	
Antibody anti-penta-His	Qiagen	34660	
Antibody anti-phospho-Akt	Cell Signaling Technology	9271	
Antibody anti-rabbit IgG H&L-HRP	Abcam	ab6721	
Antibody donkey anti-rabbit Alexa Fluor 594	ThermoFisher Scientific	A11034	
Antibody goat anti-mouse Alexa 488	ThermoFisher Scientific	A-11029	
ARPE-19 cell line	ATCC	CRL-2302	
BSA	Sigma-Aldrich	A9418-500G	
chamber culture glass slides	Corning	354118	
CytoTox-Glo Cytotoxicity Assay	Promega	G9291	
DAPI	Sigma-Aldrich	D9542-5MG	
DMEM/Ham's F12	Sigma-Aldrich	D8062	
Duo Set ELISA kit	R&D Systems	DY215-05	
EDTA	ThermoFisher Scientific	78440	
ELISAquant kit	BioProducts MD	PED613-10-Human	

Eyes (human)	Lions Gift of Sight Eye Bank (Saint Paul, MN)		
FBS	Brunschwig	P40-37500	
Fluoromount Aqueous Mounting Medium	Sigma-Aldrich	F4680-25ML	
FLUOstar Omega plate reader	BMG Labtech		
GraphPad Prism software (version 8.0)	GraphPad Software, Inc.		
GSH-Glo Glutathione Assay	Promega	V6912	
hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	Merck	107209	
ImageJ software (image processing program)	W.S. Rasband, NIH, Bethesda, MD, USA; <a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a> ; 1997–2014		
Imidazol	Axonlab	A1378.0010	
Leica DMI4000B microscope	Leica Microsystems		
LightCycler 480 Instrument II	Roche Molecular Systems		
LightCycler 480 SW1.5.1 software	Roche Molecular Systems		
NaCl	Sigma-Aldrich	71376-1000	
NaH <sub>2</sub> PO <sub>4</sub>	Axonlab	3468.1000	
Neon Transfection System	ThermoFisher Scientific	MPK5000	
Neon Transfection System 10 µL Kit	ThermoFisher Scientific	MPK1096	
Neubauer chamber	Marienfeld-superior	640010	
Ni-NTA superflow	Qiagen	30410	
Nitrocellulose	VWR	732-3197	
Omega Lum G Gel Imaging System	Aplegen Life Science		
PBS 1X	Sigma-Aldrich	D8537	
Penicillin/Streptomycin	Sigma-Aldrich	P0781-100	
PerfeCTa SYBR Green FastMix	Quantabio	95072-012	
PFA	Sigma-Aldrich	158127-100G	
Pierce BCA Protein Assay Kit	ThermoFisher Scientific	23227	
Primers	Invitrogen		See Table 1 in Supplementary Materials
pSB100X (250 ng/µL)			Mátés et al., 2009. Provide by Prof. Zsuzsanna Izsavak
pT2-CMV-GMCSF-His plasmid DNA (250 ng/µL)			Constructed using the existing pT2-CMV-PEDF-EGFP plasmid reported in Johnen, S. et al. (2012) IOVS, 53 (8), 4787-4796.
pT2-CMV-PEDF-His plasmid DNA (250 ng/µL)			Constructed using the existing pT2-CMV-PEDF-EGFP plasmid reported in Johnen, S. et al. (2012) IOVS, 53 (8), 4787-4796.
QIAamp DNA Mini Kit	QIAGEN	51304	
recombinant hGM-CSF	Peprotech	100-11	
recombinant hPEDF	BioProductsMD	004-096	
ReliaPrep RNA Cell Miniprep System	Promega	Z6011	
RIPA buffer	ThermoFisher Scientific	89901	
RNase-free DNase Set	QIAGEN	79254	
RNeasy Mini Kit	QIAGEN	74204	
SDS	Applichem	A2572	
Semi-dry transfer system for WB	Bio-Rad		
SuperMix qScript	Quantabio	95048-025	

Tris-buffered saline (TBS)	ThermoFisher Scientific	15504020	
Triton X-100	AppliChem	A4975	
Trypsin/EDTA	Sigma-Aldrich	T4174	
Tween	AppliChem	A1390	
Urea	ThermoFisher Scientific	29700	
WesternBright ECL HRP substrate	Advansta	K-12045-D50	
Whatman nitrocellulose membrane	Chemie Brunschwig	MNSC04530301	