

Materials List for:

# Investigating Protein-protein Interactions in Live Cells Using Bioluminescence Resonance Energy Transfer

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## Materials

Name	Company	Catalog Number	Comments
Nanodrop 8000	Nanodrop		Any spectrophotometer capable of reading absorbances at 260 nm will be suitable. Determining the molecular mass of the plasmid is crucial for calculating DNA quantities to be used in transfection mixes.
96-microwell plates, flat bottom, white	Greiner Bio One	655098	White plates reduce the crosstalk between wells and maximize the sensitivity of luminescence detection. Clear-bottomed wells allow monitoring of cell density. Plates must be suitable for cell culture. If using a top-reading luminometer the plate lid should be taken off.
Infinite F200Pro plate reader with control software	TECAN		Use the 'Blue 1' and 'Green 1' filters for luminescence measurement and the filter sets and dichroic mirror for GFP for fluorescence measurement. Any top-reading plate reader with capability of measuring dual-color luminescence and fluorescence is suitable.
pLuc, pYFP, positive control plasmid	N/A	N/A	Plasmids available from the authors upon request.
pGEM-3Zf(+)	Promega	P2271	Filler plasmid for equilization of DNA mass in transfection mixes. Any plasmid lacking a eukaryotic promoter would be suitable.
HEK293 cells	ECACC	85120602	Other cell lines that transfect with reasonable efficiency may be suitable.
DMEM, high glucose, with phenol red	Gibco	41966	This is the medium used for culturing HEK293 cells. Warm in 37 °C waterbath before use. If using a different cell line, replace the growth medium described here with cell-line specific medium.
DMEM, high glucose, no phenol red (substrate dilution medium)	Gibco	21063	This is the substrate dilution medium used for dilution of the luciferase substrate (EnduRen) as it does not contain phenol red, which reduces the sensitivity

			of the assay. Contains HEPES to maintain correct pH during luminescence measurements while cells are out of the CO <sub>2</sub> incubator. Warm in 37 °C waterbath before use.
OptiMEM	Gibco	31985	OptiMEM is used for dilution of GeneJuice transfection reagent. Other serum-free media would also be suitable. Warm to room temperature before use.
Fetal bovine serum	Gibco	10270	For supplementation of cell culture media at a concentration of 10% v/v.
GeneJuice transfection reagent	Novagen	70967	If using a cell line other than HEK293, it may be necessary to adjust the ratio of Genejuice transfection reagent to DNA in the transfection mixes. Other transfection reagents may be used. If using an alternative transfection reagent, it may be necessary to optimize the amount of DNA used in the transfection mixes based on manufacturer's instructions.
DMSO	Sigma	D2650	Use sterile DMSO that is suitable for tissue culture.
EnduRen live-cell substrate	Promega	E6481	Reconstitute EnduRen at 34 mg/ml in DMSO. Upon dilution of EnduRen in culture medium a precipitate may form. This will not interfere with the assay. Store reconstituted EnduRen at -20 °C, and avoid multiple freeze-thaw cycles. Ensure that reconstituted EnduRen is completely thawed before diluting it in culture medium.