

Materials List for

Bioluminescence Resonance Energy Transfer (BRET)-based Assay for Measuring Interactions of CRAF with 14-3-3 Proteins in Live Cells

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jove.com/video/66436

Materials

Name	Company	Catalog Number	Comments
Antibodies			
HaloTag® mouse monoclonal antibody	Promega	G9211	Antibody for detecting HaloTag tagged proteins by immunoblot
NanoLuc® mouse monoclonal antibody	R&D Systems	MAB10026	Antibody for detecting Nano-tagged proteins by immunoblot
CRAF mouse monoclonal antibody (E10)	Santa Crus Biotechnology	sc-7267	Antibody directly detecting CRAF proteins by immunoblot
ECL anti-mouse HRP secondary antibody	Amersham	NA931-1ML	Secondary HRP conjugated mouse antibody (from sheep)
Reagents			
X-tremeGENE™ 9	Roche/Sigma	6365809001	
NanoBRET™ kit	Promega	N1661	NanoBRET kit containing Halo 618 ligand and NanoGlo (nanoluciferase) substrate
DPBS, without Ca ⁺⁺ and Mg ⁺⁺	Quality Biologicals	114-057-101	
Trypsin-EDTA (0.05%), phenol red	Life Technologies	25300120	
DMEM cell culture media	Life Technologies	11995073	High glucose, L-glutamine, phenol red, sodium pyruvate; without HEPES, supplement media with 10% FBS, 2 mM L-glutamine and 100U penicillin-streptomycin
L-Glutamine (200 mM)	Life Technologies	25030164	
Penicillin-Streptomycin (10,000 U/mL)	Life Technologies	15140163	
Opti-MEM™ I reduced serum media	Gibco	31985062	For cell transfection
Opti-MEM reduced serum media, no phenol red	Gibco	11058021	For replating cells on Day 3. Supplement with 2 mM L-glutamine and 100U penicillin-streptomycin, along with 10% FBS (where indicated).

Invitrogen Trypan Blue Stain	Thermo Scientific	T10282	
NP40 lysis buffer	N/A	N/A	20 mM Tris (pH 8.0), 137mM NaCl, 10% glycerol, NP40 alternative (Milipore, Cat# 492016). Store at 4 degrees C.. Add the following protease and phosphatase immediately prior to use: 20 μM leupeptin, 0.5 mM sodium orthovanidate, 0.15 U/mL, 1mM PMSF.
5x gel sample buffer	N/A	N/A	240 mM Tris (pH 8.0), 9.5% SDS, 30% glycerol, 500mM DTT, 3mM bromophenol blue. Store at -20 degrees C.
Cell lines			
293FT cells (human)	Thermo Scientific	R70007	
DNA vectors			
pCMV5-Nano-CRAF WT and mutant	N/A	N/A	
pCMV5-14-3-3ζ-Halo	N/A	N/A	
Equipment			
EnVision 2104 Multimode Plate Reader	PerkinElmer 2104	2104-0010	600LP NanoBRET & M460/50 nm NanoBRET emmissions filters, Luminescence 404 mirror, 6.5 mm measurement height and 0.1 s measurement time
Invitrogen Countess™ II Automated Cell Counter	Thermo Scientific	AMQAX1000	
ThermoFisher E1-ClipTip™ Multichannel Pipettor	Thermo Scientific	4672070	
Software			
GraphPad Prism (version 10.0.3)	GraphPad	www.graphpad.com	
Other			
ThermoFisher ClipTip Multichannel pipette tips	Thermo Scientific	94410153	
Reagent Reservoir, 25 mL Divided, Sterile	Thomas Scientific	1228K16	
Perkin Elmer 384-well CulturPlate™	PerkinElmer	6007680	White, polystyrene, tissue culture treated
Countess Cell Counting Chamber Slides	Thermo Scientific	C10228	