Materials List for:

Measuring Protein Stability in Living Zebrafish Embryos Using Fluorescence Decay After Photoconversion (FDAP)

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URL: https://www.jove.com/video/52266
DOI: doi:10.3791/52266

Materials

Name	Company	Catalog Number	Comments
PyFDAP (download from the following website: http://people.tuebingen.mpg.de/mueller-lab)			Install and operate using the instructions provided on the PyFDAP website; PyFDAP is compatible with Linux, Mac, and Windows operating systems.
mMessage mMachine Sp6 Transcription Kit	Life Technologies	AM1340	To generate capped mRNA for injection into embryos
Alexa488-dextran conjugate, 3 kDa	Life Technologies	D34682	Co-inject with mRNA to create intracellular and extracellular masks
6-well plastic dish	BD Falcon		Incubate embryos in agarose-coated wells until ready for mounting
Embryo medium			250 mg/L Instant Ocean salt, 1 mg/L methylene blue in reverse osmosis water adjusted to pH 7 with $NaHCO_3$
Protease from *Streptomyces griseus*	Sigma	P5147	Make a 5 mg/ml stock and use at 1 mg/ml to dechorionate embryos at the one-cell stage
5 cm diameter glass Petri dish			For embryo dechorionation
200 ml glass beaker			For embryo dechorionation
Microinjection apparatus			For injection of mRNA and dye into embryos at the one-cell stage
Stereomicroscope			For injecting and mounting embryos
1x Danieau's medium			Dilute low melting point agarose and perform imaging in this medium; recipe: 0.2 mm filtered solution of 58 mM NaCl, 0.7 mM KCl, 0.4 mM $MgSO_4$, 0.3 mM $CaCl_2$, 5 mM HEPES pH 7.2
UltraPure low melting point agarose	Invitrogen	16520-100	For mounting embryos; use at a concentration of 1% in Danieau's medium: add 200 mg to 20 ml Danieau's medium, microwave until dissolved, then aliquot 1 ml into microcentrifuge tubes; aliquots can be stored at 4 °C, re-melted at 70 °C, and cooled to 40–42 °C when ready to use

Glass Pasteur pipette	Kimble Chase (via Fisher)	63A53WT	For mounting embryos; flame the tip to prevent jagged edges from injuring embryos
Metal probe			For positioning embryos during mounting
Glass bottom dishes	MatTek	P35G-1.5-14-C	Use the appropriate cover glass thickness for your objective; part number listed here is for cover glass No. 1.5
15 ml tube filled with ~5 ml embryo medium	BD Falcon		For rinsing residual agarose from the Pasteur pipette
Inverted laser scanning confocal microscope			A mercury arc lamp, 488 nm laser, 543 nm laser, and the appropriate filter sets are required
Heated stage			To maintain embryos at the optimal temperature of 28 °C during the experiment
Confocal software capable of time-lapse imaging			Must be able to define multiple positions and automatically image them at defined intervals
25X or 40X water objective			Objective for imaging
10X air objective			Objective for photoconversion
Immersion oil			Immersion oil with the same refractive index as water