Montana Molecular
Fluorescent Biosensors for Live Cell Discovery

Red Dimerization Dependent Sensor for cAMP

This genetically-encoded sensor is in a CMV expression plasmid. The tube is labeled with DNA concentration.

STEP 1: Transform E. coli with 100ng of plasmid DNA according to standard protocol.
STEP 2: Midi prep or Maxi prep DNA. We use Qiagen HiSpeed Midi prep kit.

Our red cAMP sensor decreases in fluorescence following receptor activation and increasing cAMP concentration.

Coating 96-Well Plates with Poly-D-Lysine

Recommended 96-well plates

- Greiner Cell-Coat polystyrene plates for use on fluorescence plate reader
- Greiner glass bottom for live cell imaging.

1. Make a working solution of 25 ug/ml solution of Poly-D-Lysine (PDL) using sterile H20.
2. Add 70 ul of PDL working solution per well and incubate at room temperature for 1 hr (coating concentration is approximately 5 ug/cm2).
3. Discard PDL and rinse wells once with 100 uL sterile H_20.
4. Allow to dry and proceed to plating cells.

Plating and Transfecting HEK293T cells on 96-well plates.

1. One day before transfection, plate cells in 100 µl EMEM (avail. from ATCC) growth medium per well **without antibiotics** so that the cells will be 90-95% confluent at the time of transfection (approximately 24 hours later). If imaging on 96 well plates, PDL-coated plates are highly recommended. See above for plates to use and the PDL coating protocol.
2. For each transient transfection sample/rxn (i.e. one well in a 96-well plate), prepare complex as as follows:
   a) Dilute DNA in 25 µl of Opti-MEM (avail. from Life Technologies). Mix gently. For imaging applications, add 60 ng of sensor plasmid DNA plus 40 ng of receptor plasmid DNA, if co-transfecting a receptor.
   b) Mix Lipofectamine 2000 (avail. from Life Technologies) gently before use, then dilute the appropriate amount in 25 µl of Opti-MEM. We use a 1:3 DNA:Lipofect-

amine ratio, so add .3-.42 ul Lipofectamine. Incubate for 5 minutes at room temperature. Note: Proceed to Step c within 25 minutes.

c) After the 5 minute incubation, combine the diluted DNA/Opti-mem mixture (from step A) with diluted Lipofectamine 2000 (total volume = 50 µl). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy). Note: Complexes are stable for 6 hours at room temperature.

3. Add the 50 µl rxn mix to the well (containing cells and 100 µl medium). Mix gently by rocking the plate back and forth.
4. Incubate cells at 37°C in the CO2 incubator. Remove transfection mix after 4-6 hours and replace with fresh medium.
5. Continue to incubate cells at 37°C in the CO2 incubator for 24-48 hours prior to testing/ screening.
6. Immediately prior to screening, replace media with 100 µl of pre-warmed 1X DPBS per well.

<u>Excitation and Emission WaveLengths</u>

We use 572/20 nm excitation and 630/30 nm emission to collect the red fluorescence from the sensor.

Cell Imaging

At Montana Molecular, our imaging system is a Zeiss Axiovert S100TV inverted microscope fitted with computer controlled excitation/emission filter wheels, shutters, and a Qimaging Retiga Exi CCD camera.

Cells are imaged live at 25°C using the 10X objective lens. Use 572/20 nm excitation and 630/30 nm emission filters to collect the red fluorescence from the red cAMP sensor.

Image Acquisition/Analysis

Montana Molecular uses iVision software from BioVision Technologies for image acquisition and analysis. For cell imaging, a minimum of 20 images are acquired (binning 2x2), with exposure times of .2 to .5 milliseconds and 5 seconds in between frames. Drug is typically added after frame 7.

To analyze image stacks, regions of interest are defined around individual cells and the mean pixel value of the cell is obtained for each frame. Multiple cells are analyzed and averaged. Delta F/F values are calculated using the frame immediately before drug addition and the frame 15-20 seconds post drug. The background fluorescence is defined as a region of the image that contains no cells. The average value of this region is subtracted frame by frame from the measurements of the mean pixel values of the fluorescent cells.

Thank you for ordering! Please let us know if you have any questions about these protocols and how the sensors worked for you. We are here to help.

Please visit www.montanamolecular.com to contact us and read more about our fluorescent sensors and live cell assays.