

# Multiplexing Cell Based Assays in HTS

Kelly Cassutt, Al McGrath, Masanobu Fujiwara, and Shouming Du / Hamamatsu Photonics Corporation 360 Foothill Road, Bridgewater, NJ 08807

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## Abstract:

The FDSS6000 from Hamamatsu uses Xenon lamps, enabling the use of fluorescent dyes ranging from UV to visible excitation wavelengths. The FDSS6000 can record up to two excitation wavelengths and two emission wavelengths in the same assay. Current screening strategies use only one dye per assay, such as Fura-2AM, Fluo-3AM, or Fluo-4AM (calcium mobilization), or DisBAC<sub>2</sub>(3) (membrane potential). We present a novel multiplexing method for HTS Screening: Loading the same cells using both the UV dye Fura-2AM and a no wash Membrane Potential Dye. Multiplexing assays measure orthogonal cell responses (calcium mobilization and membrane potential) using one volume of valuable library compound.

## Introduction:

Several groups have reported results using double dye loaded (multiplexed) cells for studying either calcium channels or G Protein Coupled receptors, including Fura-2 AM/DisBac<sub>2</sub>(3)<sup>1</sup>, Indo-1AM/DisBac<sub>2</sub>(3)<sup>2</sup>, Fura-2 AM/DiBac<sub>4</sub>(3)<sup>3</sup>, and Fluo-4AM/Di4 ANEPPS<sup>4</sup>. These assays used single channel Spectrofluorometers for readout.

We report how multiplexing identifies off-receptor agonism but that desensitizing 'off-receptors' eliminates target receptor agonism using calcium mobilization readout but not membrane potential readout.

## Materials and Methods:

Nonadherent cells endogenously expressing receptors, 1x10<sup>7</sup>/mL, were loaded using 1.2 μM Fura-2 AM for 1h at room temp. Cells were diluted to 1.1 X 10<sup>6</sup>/mL in No Wash Membrane Potential Dye, 0.25 X final concentration and aliquotted to 384 wells (Figure 1). Following a 15 minute incubation cells were read on the FDSS6000. For Membrane Potential Dye the excitation:emission wavelengths were 480:540; for Fura-2AM wavelengths were 380:540 (fluorescence decreases upon calcium binding). Data was analyzed using CeuticalSoft (Hudson, NY).

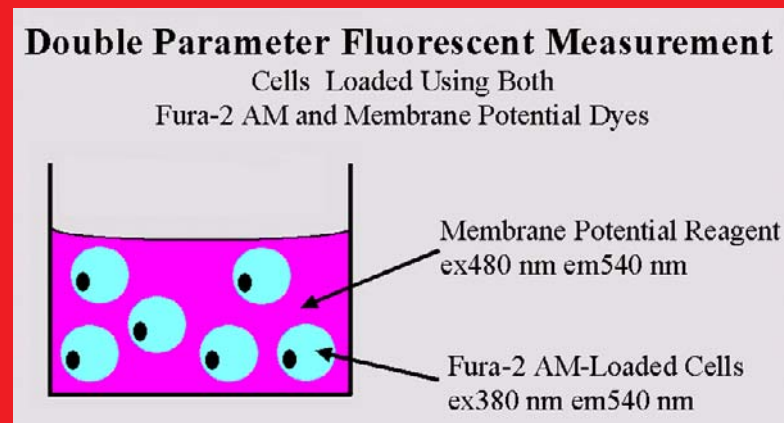


Figure 1. Cells are double dye loaded using both Fura-2AM and No Wash Membrane Potential Dye.

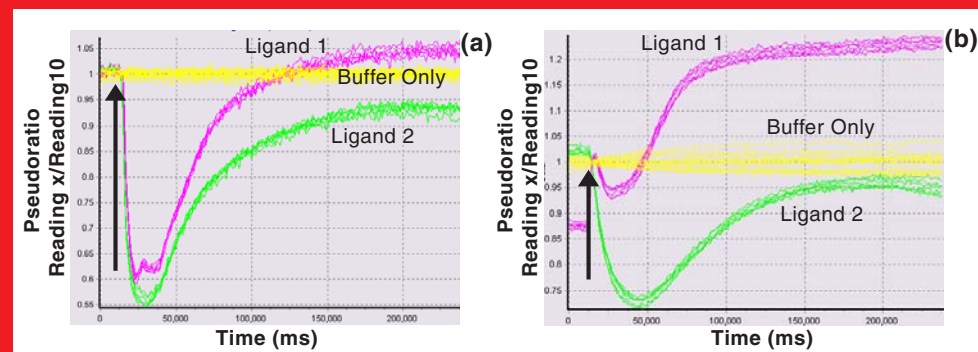


Figure 2. Fluorescence of calcium mobilization (a) and membrane potential (b). Arrows indicate injection point. Note that Ligand 1 induces calcium mobilization and membrane potential depolarization; Ligand 2 induces calcium mobilization and membrane potential hyperpolarization.

Ligand	EC <sub>50</sub> (μM)	
	Calcium Mobilization	Membrane Potential
1	12	126
2	0.4	0.3

Table 1. Agonist EC<sub>50</sub> from two ligands, two readouts (calcium vs. membrane potential). Note the 10 fold difference in potency of Ligand 1 related to readout, as compared to Ligand 2.

## ASSAY FAILURE-NO INHIBITION USING LIGAND 1 AGAINST KNOWN ANTAGONISTS

PLAN B: Desensitize extraneous receptors using Ligand 2

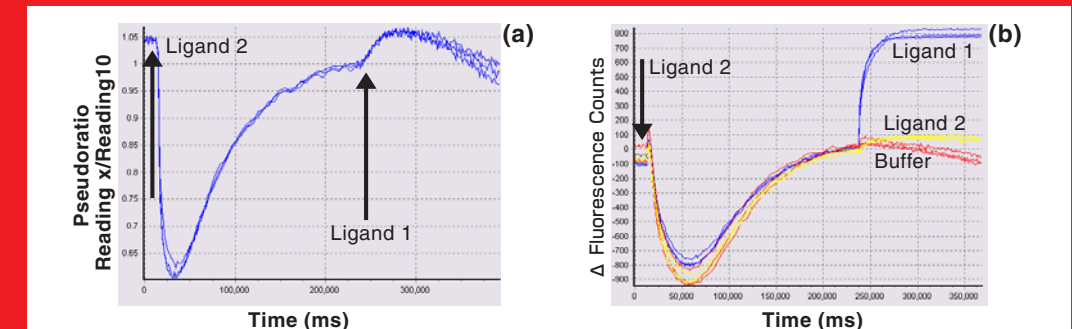


Figure 3. Fluorescence of calcium mobilization (a) and membrane potential (b). Arrows indicate injection point. Ligand 2 eliminates Ligand 1-mediated calcium mobilization but not membrane depolarization.

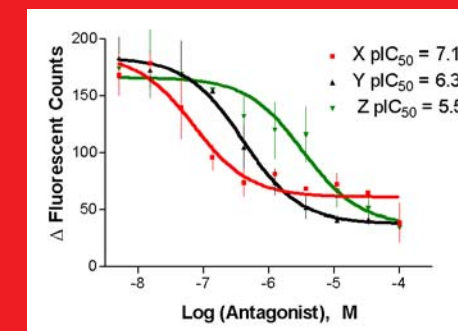


Figure 4. Membrane depolarization, antagonist IC<sub>50</sub> curves using Ligand 2 desensitization protocol (see Figure 3(b)). Ligand 1 EC<sub>50</sub> is 178 μM.

## Summary:

Multiplexing a Calcium Mobilization Dye with Membrane Potential Dye in the same cells provides orthogonal results. Calcium mobilization predicts neither membrane depolarization nor hyperpolarization. Ligand desensitization did not work for calcium mobilization readout but did for membrane potential readout.

## References:

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360 Foothill Road, P.O. Box 6910, Bridgewater, NJ 08807  
Phone: 908-231-1116 / Fax: 908-231-0852 / E-mail: syssales@hamamatsu.com

[www.fdssdrug.com](http://www.fdssdrug.com)