

## Technical Data Sheet

**Codex ACTOne™ Non-Wash Calcium Dye Kit**

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**Product Information**

<b>Catalog Number:</b>	CB-80500-301
<b>Size:</b>	Reagents for 10 plates
<b>Components:</b>	Codex Calcium Dye, 10 vials, lyophilized (Part No: 80500-110) 10X Calcium Dye Signal Enhancer, 10 ml (Part No: 80500-112)

**Description**

The Codex ACTOne™ Non-Wash Calcium Dye Kit allows homogeneous measurement for identifying cardiotoxic compounds using iPSC-CMs. It is a fast, simple, and reliable fluorescence-based method. The dye showed very little effect on beating rate and peak amplitude over a very long period. The dye can be used to quantify and report drug-induced early-after depolarization (EAD)-like waveforms, cardiomyocyte ectopic beats, changes in beating rate, and peak amplitude from a variety of agents. It is user friendly and cost effective. The assay can be easily implemented in a high throughput environment.

**Storage**

Codex Calcium Dye	-20°C (protected from light)
10X Calcium Dye Signal Enhancer	Room Temp.

**Materials not included**

DMSO	Sigma D4540
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**ASSAY PROTOCOL****Prepare the cell plate:**

1. Human iPSC-CMs from Codex BioSolutions were plated in 96-well plates that had been pre-coated with fibronectin overnight at 37 °C. The cell density is 60,000 cells/well.
2. Cells were cultured for 24 h in plating media and, after that, switched to the maintenance media. The maintenance media was changed every other day and 24 h prior to the day of the experiment.
3. Cells were tested 2 weeks after plating at which time they formed a spontaneously beating monolayer.

**Prepare the Dye Loading Solution:**

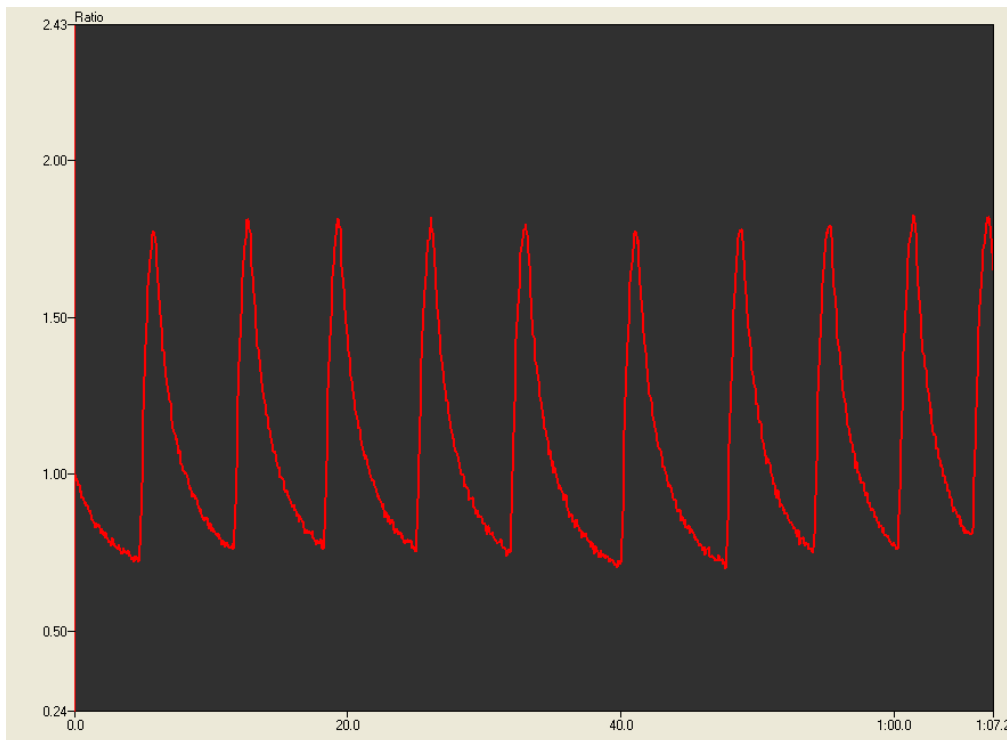
On the 2<sup>nd</sup> day:

1. Prepare Buffer A (1X HBSS with 20 mM HEPES):  
10 ml of 1M HEPES, pH 7.3 + 490 ml of 1X HBSS
2. Prepare stock solution of calcium dye  
Add 8µl of DMSO into each well containing 50 µg of calcium dye
3. Prepare **2X Dye Loading Solution** (1 plates).  
Add 0.8 ml of Codex 10X Calcium Dye Signal Enhancer into 7.2 ml of Buffer A.  
Add 8 µl of calcium dye stock solution. Mix well by vortexing.

## Assay:

1. Take the cell plate out from the incubator.
2. Add same volume of **2X Dye Loading Solution** into each well, 80  $\mu$ l to a 96-well plate
3. Incubate at 37 °C incubator for 1 hr.
3. Place the cell plates on a FDSS or FLIPR plate form and Ca<sup>2+</sup> simultaneous transient signals were collected from 96-well plates. The standard filters for calcium assays (same as Fluo-4) is used

## Appendix



**Figure 1.** Human iPSC-CMs from Codex BioSolutions were plated in 96-well plates that had been pre-coated with fibronectin overnight at 37 °C. The cell density is 60,000 cells/well. Cells were cultured for 24 h in plating media and, after that, switched to the maintenance media. The maintenance media was changed every other day and 24 h prior to the day of the experiment. Cells were tested 2 weeks after plating at which time they formed a spontaneously beating monolayer. On experiment day, cells were incubated with Codex ACTOne® dye for 1 h at 37 °C, 5% CO<sub>2</sub>. FDSS7000 imaging platform (Hamamatsu Ltd., Hamamatsu, Japan) was used to simultaneously collected Ca<sup>2+</sup> transient signals from 96-well plates, at a sampling rate of 20 Hz.

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