Technical Data Sheet

# Codex ACTOne™ Non-Wash Calcium Dye Kit

## Product Information

**Catalog Number:** CB-80500-311
**Size**: Reagents for 100 plates
**Components:** Codex Calcium Dye, 100 vials, lyophilized (Part No: 80500-110)
10X Calcium Dye Signal Enhancer, 100 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 |

## 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 2nd 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.

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## Assay:

1. Take the cell plate out from the incubator.

2. Add same volume of **2X Dye Loading Solution** into each well, 80 μl 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 Ca2+ simultaneous transient signals were collected from 96-well plates. The standard filters for calcium assays (same as Fluo-4) is used

# Appendix

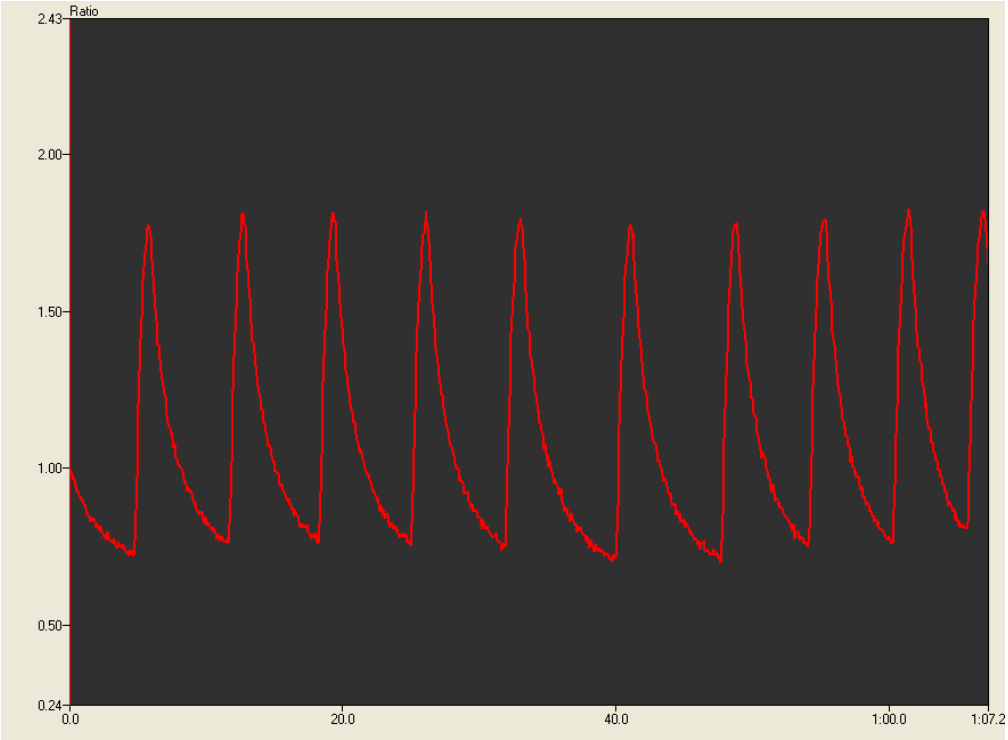


**Figure 1.** Human iPSC-CMs from Codex BioSolutions were plated in 96-well plates that had been pre-coated with fibronectin overnight at 37 °C. The cell density is 60,000 cells/well. Cells were cultured for 24 h in plating media and, after that, switched to the maintenance media. The maintenance media 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% CO2. FDSS7000 imaging platform (Hamamatsu Ltd., Hamamatsu, Japan) was used to simultaneously collected Ca2+ transient signals from 96-well plates, at a sampling rate of 20 Hz.



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