**Substance:** OSU6162

**Date:** 160822

**Zebrafish DOB:** 160812 (10 dpf)

**Aim:** Dose response OSU6162/ light/dark

**Volume in wells:** 1 ml + 20 µl

**Stock OSU6162 solution:** 10 mM in DMSO (50 µl)

**Dilute to:** **500 µM** (50 µl 10 mM OSU6162 + 950 µl E3)

**150 µM** (300 µl 500 µM OSU6162 + 700 µl E3)

**50 µM** (100 µl 500 µM OSU6162 + 900 µl E3)

**10 µM OSU6162:** (20 µl 500 µM OSU6162) / **3 µM OSU6162:** (20 µl 150 µM OSU6162) / **1 µM OSU6162:** (20 µl 50 µM OSU6162)

**Control:** 20 µl 5% DMSO/E3 (0.2% in wells)

**PTZ solution:** 50 mM in E3 (50 µl to each well)

Plate 1:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| A | OSU6162 3 µM | OSU6162 3 µM | OSU6162 3 µM | OSU6162 3 µM | OSU6162 3 µM | OSU6162 3 µM |
| B | OSU6162 10 µM | OSU6162 10 µM | OSU6162 10 µM | OSU6162 10 µM | OSU6162 10 µM | OSU6162 10 µM |
| C | Control | Control | Control | Control | Control | Control |
| D | OSU6162 1 µM | OSU6162 1 µM | OSU6162 1 µM | OSU6162 1 µM | OSU6162 1 µM | OSU6162 1 µM |

Plate 2:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| E | OSU6162 3 µM | OSU6162 3 µM | OSU6162 3 µM | OSU6162 3 µM | OSU6162 3 µM | OSU6162 3 µM |
| F | OSU6162 10 µM | OSU6162 10 µM | OSU6162 10 µM | OSU6162 10 µM | OSU6162 10 µM | OSU6162 10 µM |
| G | Control | Control | Control | Control | Control | Control |
| H | OSU6162 1 µM | OSU6162 1 µM | OSU6162 1 µM | OSU6162 1 µM | OSU6162 1 µM | OSU6162 1 µM |

File

**Add OSU6162**

**Record during 13x5 min in light/dark**

After 0 min: recording during 5 min (light) 123442

After 5 min: recording during 5 min (light) 123944

After 10 min: recording during 5 min (dark) 124445

After 15 min: recording during 5 min (dark) 124947

After 20 min: recording during 5 min (light) 125448

After 25 min: recording during 5 min (light) 125950

After 30 min: recording during 5 min (dark) 130451

After 35 min: recording during 5 min (dark) 130953

After 40 min: recording during 5 min (light) 131454

After 45 min: recording during 5 min (light) 131956

After 50 min: recording during 5 min (dark) 132458

After 55 min: recording during 5 min (dark) 133000

After 60 min: recording during 5 min (light) 133502

**Add PTZ (2.5 mM) to all wells**

**Record during 13x5 min in total darkness**

After 0 min: recording during 5 min 134745

After 5 min: recording during 5 min 135247

After 10 min: recording during 5 min 135749

After 15 min: recording during 5 min 140251

After 20 min: recording during 5 min 140753

After 25 min: recording during 5 min 141255

After 30 min: recording during 5 min 141757

After 35 min: recording during 5 min 142259

After 40 min: recording during 5 min 142801

After 45 min: recording during 5 min 143303

After 50 min: recording during 5 min 143806

After 55 min: recording during 5 min 144308

After 60 min: recording during 5 min 144810

**Other conditions:** Cover blanket, light on/off

Exposure: 1350 µsec, 300 s per recording

Thermostat: 31.5°C

Water temperature: in bath: 30.5°C in wells: 28.0°C

After experiment: all larvae terminated on ice