**Substance:** PCAP1

**Date:** 160519

**Zebrafish DOB:** 160509(10 dpf)

**Aim:** Dose response PCAP1/ light/dark

**Volume in wells:** 1 ml + 20 µl

**Stock PCAP1 solution:** 10 mM in DMSO (50 µl)

**Dilute to:** **500 µM** (50 µl 10 mM PCAP1 + 950 µl E3)

**150 µM** (300 µl 500 µM PCAP1 + 700 µl E3)

**50 µM** (100 µl 500 µM PCAP1 + 900 µl E3)

**10 µM PCAP1:** (20 µl 500 µM PCAP1) / **3 µM PCAP1:** (20 µl 150 µM PCAP1) / **1 µM PCAP1:** (20 µl 50 µM PCAP1)

**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 | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM |
| B | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM |
| C | Control | Control | Control | Control | Control | Control |
| D | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM |

Plate 2:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| E | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM |
| F | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM |
| G | Control | Control | Control | Control | Control | Control |
| H | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM |

File

**Add PCAP1**

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

After 0 min: recording during 5 min (light) 105828

After 5 min: recording during 5 min (light) 110330

After 10 min: recording during 5 min (dark) 110831

After 15 min: recording during 5 min (dark) 111333

After 20 min: recording during 5 min (light) 111834

After 25 min: recording during 5 min (light) 112336

After 30 min: recording during 5 min (dark) 112837

After 35 min: recording during 5 min (dark) 113339

After 40 min: recording during 5 min (light) 113840

After 45 min: recording during 5 min (light) 114342

After 50 min: recording during 5 min (dark) 114844

After 55 min: recording during 5 min (dark) 115346

After 60 min: recording during 5 min (light) 115848

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

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

After 0 min: recording during 5 min 121211

After 5 min: recording during 5 min 121713

After 10 min: recording during 5 min 122215

After 15 min: recording during 5 min 122716

After 20 min: recording during 5 min 123218

After 25 min: recording during 5 min 123719

After 30 min: recording during 5 min 124221

After 35 min: recording during 5 min 124723

After 40 min: recording during 5 min 125224

After 45 min: recording during 5 min 125726

After 50 min: recording during 5 min 130228

After 55 min: recording during 5 min 130730

After 60 min: recording during 5 min 131232

**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