**Substance:** PCAP2

**Date:** 160415

**Zebrafish DOB:** 160405(10 dpf)

**Aim:** Dose response PCAP2/ light/dark

**Volume in wells:** 1 ml + 20 µl

**Stock PCAP2 solution:** 10 mM in DMSO (50 µl)

**Dilute to:** **500 µM** (50 µl 10 mM PCAP2 + 950 µl E3)

**150 µM** (300 µl 500 µM PCAP2 + 700 µl E3)

**50 µM** (100 µl 500 µM PCAP2 + 900 µl E3)

**10 µM PCAP2:** (20 µl 500 µM PCAP2) / **3 µM PCAP2:** (20 µl 150 µM PCAP2) / **1 µM PCAP2:** (20 µl 50 µM PCAP2)

**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 | Control | Control | Control | Control | Control | Control |
| B | PCAP2 1 µM | PCAP2 1 µM | PCAP2 1 µM | PCAP2 1 µM | PCAP2 1 µM | PCAP2 1 µM |
| C | PCAP2 3 µM | PCAP2 3 µM | PCAP2 3 µM | PCAP2 3 µM | PCAP2 3 µM | PCAP2 3 µM |
| D | PCAP2 10 µM | PCAP2 10 µM | PCAP2 10 µM | PCAP2 10 µM | PCAP2 10 µM | PCAP2 10 µM |

Plate 2:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| E | Control | Control | Control | Control | Control | Control |
| F | PCAP2 1 µM | PCAP2 1 µM | PCAP2 1 µM | PCAP2 1 µM | PCAP2 1 µM | PCAP2 1 µM |
| G | PCAP2 3 µM | PCAP2 3 µM | PCAP2 3 µM | PCAP2 3 µM | PCAP2 3 µM | PCAP2 3 µM |
| H | PCAP2 10 µM | PCAP2 10 µM | PCAP2 10 µM | PCAP2 10 µM | PCAP2 10 µM | PCAP2 10 µM |

File

**Add PCAP2**

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

After 0 min: recording during 5 min (light) 133427

After 5 min: recording during 5 min (light) 133928

After 10 min: recording during 5 min (dark) 134430

After 15 min: recording during 5 min (dark) 134931

After 20 min: recording during 5 min (light) 135433

After 25 min: recording during 5 min (light) 135934

After 30 min: recording during 5 min (dark) 140436

After 35 min: recording during 5 min (dark) 140939

After 40 min: recording during 5 min (light) 141441

After 45 min: recording during 5 min (light) 141943

After 50 min: recording during 5 min (dark) 142445

After 55 min: recording during 5 min (dark) 142947

After 60 min: recording during 5 min (light) 143449

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

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

After 0 min: recording during 5 min 144432

After 5 min: recording during 5 min 144934

After 10 min: recording during 5 min 145435

After 15 min: recording during 5 min 145937

After 20 min: recording during 5 min 150439

After 25 min: recording during 5 min 150940

After 30 min: recording during 5 min 151442

After 35 min: recording during 5 min 151944

After 40 min: recording during 5 min 152445

After 45 min: recording during 5 min 152947

After 50 min: recording during 5 min 153449

After 55 min: recording during 5 min 153951

After 60 min: recording during 5 min 154453

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

Exposure: 1360 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