**Substance:** PCAP814

**Date:** 160609

**Zebrafish DOB:** 160530 (10 dpf)

**Aim:** Dose response PCAP814/ light/dark

**Volume in wells:** 1 ml + 20 µl

**Stock PCAP814 solution:** 10 mM in DMSO (50 µl)

**Dilute to:** **500 µM** (50 µl 10 mM PCAP814 + 950 µl E3)

**150 µM** (300 µl 500 µM PCAP814 + 700 µl E3)

**50 µM** (100 µl 500 µM PCAP814 + 900 µl E3)

**10 µM PCAP814:** (20 µl 500 µM PCAP814) / **3 µM PCAP814:** (20 µl 150 µM PCAP814) / **1 µM PCAP814:** (20 µl 50 µM PCAP814)

**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 | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM |
| B | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM |
| C | Control | Control | Control | Control | Control | Control |
| D | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM |

Plate 2:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| E | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM |
| F | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM |
| G | Control | Control | Control | Control | Control | Control |
| H | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM |

File

**Add PCAP814**

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

After 0 min: recording during 5 min (light) 104624

After 5 min: recording during 5 min (light) 105126

After 10 min: recording during 5 min (dark) 105627

After 15 min: recording during 5 min (dark) 110128

After 20 min: recording during 5 min (light) 110638

After 25 min: recording during 5 min (light) 111140

After 30 min: recording during 5 min (dark) 111642

After 35 min: recording during 5 min (dark) 112144

After 40 min: recording during 5 min (light) 112647

After 45 min: recording during 5 min (light) 113149

After 50 min: recording during 5 min (dark) 113651

After 55 min: recording during 5 min (dark) 114153

After 60 min: recording during 5 min (light) 114655

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

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

After 0 min: recording during 5 min 115704

After 5 min: recording during 5 min 120206

After 10 min: recording during 5 min 120707

After 15 min: recording during 5 min 121209

After 20 min: recording during 5 min 121710

After 25 min: recording during 5 min 122212

After 30 min: recording during 5 min 122714

After 35 min: recording during 5 min 123215

After 40 min: recording during 5 min 123717

After 45 min: recording during 5 min 124219

After 50 min: recording during 5 min 124721

After 55 min: recording during 5 min 125223

After 60 min: recording during 5 min 125725

**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