**Substance:** PCAP814

**Date:** 160602

**Zebrafish DOB:** 160523 (10 dpf)

**Aim:** Dose response PCAP814/ light

**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)

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

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

**25 µM** (60 µl 500 µM Apomorphine + 1140 µl E3)

Plate 1:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| A | Control | Control | Control | Control | Control | Control |
| B | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM | PCAP814 1 µM |
| C | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM | PCAP814 3 µM |
| D | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM | PCAP814 10 µM |

Plate 2:

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

File

**Add PCAP814**

**Record during 13x5 min in light**

After 0 min: recording during 5 min 110652

After 5 min: recording during 5 min 111154

After 10 min: recording during 5 min 111655

After 15 min: recording during 5 min 112157

After 20 min: recording during 5 min 112658

After 25 min: recording during 5 min 113200

After 30 min: recording during 5 min 113701

After 35 min: recording during 5 min 114203

After 40 min: recording during 5 min 114704

After 45 min: recording during 5 min 115206

After 50 min: recording during 5 min 115707

After 55 min: recording during 5 min 120209

After 60 min: recording during 5 min 120710

**Add Apomorphine (0.5 µM) to all wells**

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

After 0 min: recording during 5 min 121740

After 5 min: recording during 5 min 122242

After 10 min: recording during 5 min 122744

After 15 min: recording during 5 min 123245

After 20 min: recording during 5 min 123747

After 25 min: recording during 5 min 124248

After 30 min: recording during 5 min 124750

After 35 min: recording during 5 min 125252

After 40 min: recording during 5 min 125754

After 45 min: recording during 5 min 130255

After 50 min: recording during 5 min 130757

After 55 min: recording during 5 min 131259

After 60 min: recording during 5 min 131801

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

Exposure: 1340 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