**Substance:** PCAP814

**Date:** 160602

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

**Aim:** Dose response PCAP814/ 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)

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

**Dilute to: 2500 µM** (300 µl 10 mM Apomorphine + 900 µl E3)

Plate 1:

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

Plate 2:

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

File

**Add PCAP814**

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

After 0 min: recording during 5 min 144825

After 5 min: recording during 5 min 145327

After 10 min: recording during 5 min 145828

After 15 min: recording during 5 min 150330

After 20 min: recording during 5 min 150831

After 25 min: recording during 5 min 151333

After 30 min: recording during 5 min 151834

After 35 min: recording during 5 min 152336

After 40 min: recording during 5 min 152837

After 45 min: recording during 5 min 153339

After 50 min: recording during 5 min 153841

After 55 min: recording during 5 min 154343

After 60 min: recording during 5 min 154845

**Add Apomorphine (50 µM) to all wells**

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

After 0 min: recording during 5 min 155737

After 5 min: recording during 5 min 160241

After 10 min: recording during 5 min 160753

After 15 min: recording during 5 min 161301

After 20 min: recording during 5 min 161813

After 25 min: recording during 5 min 162321

After 30 min: recording during 5 min 162832

After 35 min: recording during 5 min 163338

After 40 min: recording during 5 min 163851

After 45 min: recording during 5 min 164354

After 50 min: recording during 5 min 164857

After 55 min: recording during 5 min 165359

After 60 min: recording during 5 min 165902

**Other conditions:** Cover blanket, light off (IR-light only)

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