**Substance:** PCAP2

**Date:** 160415

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

**Aim:** Dose response PCAP2/ 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)

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

Plate 2:

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

File

**Add PCAP2**

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

After 0 min: recording during 5 min 120155

After 5 min: recording during 5 min 120656

After 10 min: recording during 5 min 121157

After 15 min: recording during 5 min 121659

After 20 min: recording during 5 min 122200

After 25 min: recording during 5 min 122702

After 30 min: recording during 5 min 123203

After 35 min: recording during 5 min 123705

After 40 min: recording during 5 min 124206

After 45 min: recording during 5 min 124708

After 50 min: recording during 5 min 125209

After 55 min: recording during 5 min 125711

After 60 min: recording during 5 min 130212

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

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

After 0 min: recording during 5 min 131233

After 5 min: recording during 5 min 131735

After 10 min: recording during 5 min 132237

After 15 min: recording during 5 min 132738

After 20 min: recording during 5 min 133240

After 25 min: recording during 5 min 133742

After 30 min: recording during 5 min 134243

After 35 min: recording during 5 min 134745

After 40 min: recording during 5 min 135247

After 45 min: recording during 5 min 135748

After 50 min: recording during 5 min 140250

After 55 min: recording during 5 min 140752

After 60 min: recording during 5 min 141254

**Other conditions:** Cover blanket, in total darkness (IR-light only)

Exposure: 1380 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