**Substance:** PCAP1

**Date:** 160422

**Zebrafish DOB:** 160412 (10 dpf)

**Aim:** Dose response PCAP1/ light

**Volume in wells:** 1 ml + 20 µl

**Stock PCAP1 solution:** 10 mM in DMSO (50 µl)

**Dilute to:** **500 µM** (50 µl 10 mM PCAP1 + 950 µl E3)

**150 µM** (300 µl 500 µM PCAP1 + 700 µl E3)

**50 µM** (100 µl 500 µM PCAP1 + 900 µl E3)

**10 µM PCAP1:** (20 µl 500 µM PCAP1) / **3 µM PCAP1:** (20 µl 150 µM PCAP1) / **1 µM PCAP1:** (20 µl 50 µM PCAP1)

**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** (50 µl 500 µM Apomorphine + 950 µl E3)

Plate 1:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| A | Control | Control | Control | Control | Control | Control |
| B | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM |
| C | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM |
| D | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM |

Plate 2:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| E | Control | Control | Control | Control | Control | Control |
| F | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM | PCAP1 1 µM |
| G | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM | PCAP1 3 µM |
| H | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM | PCAP1 10 µM |

File

**Add PCAP1**

**Record during 13x5 min in light**

After 0 min: recording during 5 min 110230

After 5 min: recording during 5 min 110732

After 10 min: recording during 5 min 111234

After 15 min: recording during 5 min 111737

After 20 min: recording during 5 min 112240

After 25 min: recording during 5 min 112742

After 30 min: recording during 5 min 113244

After 35 min: recording during 5 min 113747

After 40 min: recording during 5 min 114249

After 45 min: recording during 5 min 114752

After 50 min: recording during 5 min 115254

After 55 min: recording during 5 min 115757

After 60 min: recording during 5 min 120259

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

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

After 0 min: recording during 5 min 121154

After 5 min: recording during 5 min 121657

After 10 min: recording during 5 min 122200

After 15 min: recording during 5 min 122702

After 20 min: recording during 5 min 123205

After 25 min: recording during 5 min 123708

After 30 min: recording during 5 min 124210

After 35 min: recording during 5 min 124713

After 40 min: recording during 5 min 125216

After 45 min: recording during 5 min 125719

After 50 min: recording during 5 min 130222

After 55 min: recording during 5 min 130725

After 60 min: recording during 5 min 131228

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

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