**Substance:** CNO

**Date:** 160321

**Zebrafish DOB:** 160311 (10 dpf)

**Aim:** Dose response CNO/ light

**Volume in wells:** 1 ml + 20 µl

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

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

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

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

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

**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 | CNO 1 µM | CNO 1 µM | CNO 1 µM | CNO 1 µM | CNO 1 µM | CNO 1 µM |
| C | CNO 3 µM | CNO 3 µM | CNO 3 µM | CNO 3 µM | CNO 3 µM | CNO 3 µM |
| D | CNO 10 µM | CNO 10 µM | CNO 10 µM | CNO 10 µM | CNO 10 µM | CNO 10 µM |

Plate 2:

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

File

**Add CNO**

**Record during 13x5 min in light**

After 0 min: recording during 5 min 120458

After 5 min: recording during 5 min 120959

After 10 min: recording during 5 min 121501

After 15 min: recording during 5 min 122002

After 20 min: recording during 5 min 122504

After 25 min: recording during 5 min 123005

After 30 min: recording during 5 min 123507

After 35 min: recording during 5 min 124008

After 40 min: recording during 5 min 124510

After 45 min: recording during 5 min 125011

After 50 min: recording during 5 min 125513

After 55 min: recording during 5 min 130014

After 60 min: recording during 5 min 130516

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

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

After 0 min: recording during 5 min 131724

After 5 min: recording during 5 min 132226

After 10 min: recording during 5 min 132728

After 15 min: recording during 5 min 133229

After 20 min: recording during 5 min 133731

After 25 min: recording during 5 min 134233

After 30 min: recording during 5 min 134734

After 35 min: recording during 5 min 135236

After 40 min: recording during 5 min 135738

After 45 min: recording during 5 min 140240

After 50 min: recording during 5 min 140741

After 55 min: recording during 5 min 141243

After 60 min: recording during 5 min 141745

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

Exposure: 1350 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