**Substance:** CNO

**Date:** 160324

**Zebrafish DOB:** 160314 (10 dpf)

**Aim:** Dose response CNO/ light/dark

**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)

**PTZ solution:** 50 mM in E3 (50 µl to each well)

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/dark**

After 0 min: recording during 5 min (light) 104456

After 5 min: recording during 5 min (light) 104958

After 10 min: recording during 5 min (dark) 105500

After 15 min: recording during 5 min (dark) 110001

After 20 min: recording during 5 min (light) 110503

After 25 min: recording during 5 min (light) 111004

After 30 min: recording during 5 min (dark) 111506

After 35 min: recording during 5 min (dark) 112007

After 40 min: recording during 5 min (light) 112509

After 45 min: recording during 5 min (light) 113010

After 50 min: recording during 5 min (dark) 113512

After 55 min: recording during 5 min (dark) 114013

After 60 min: recording during 5 min (light) 114515

**Add PTZ (2.5 mM) to all wells**

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

After 0 min: recording during 5 min 115931

After 5 min: recording during 5 min 120432

After 10 min: recording during 5 min 120934

After 15 min: recording during 5 min 121436

After 20 min: recording during 5 min 121937

After 25 min: recording during 5 min 122439

After 30 min: recording during 5 min 122940

After 35 min: recording during 5 min 123442

After 40 min: recording during 5 min 123944

After 45 min: recording during 5 min 124446

After 50 min: recording during 5 min 124947

After 55 min: recording during 5 min 125449

After 60 min: recording during 5 min 125951

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

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