**Substance:** NDMC

**Date:** 160317

**Zebrafish DOB:** 160307 (10 dpf)

**Aim:** Dose response NDMC/ light

**Volume in wells:** 1 ml + 20 µl

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

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

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

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

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

**Control:** 20 µl 5% DMSO/E3 (0.2% in wells)

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

**Dilute to: 2500 µM** (50 µl 10 mM Apomorphine + 150 µl E3)

Plate 1:

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

Plate 2:

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

File

**Add NDMC**

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

After 0 min: recording during 5 min 142255

After 5 min: recording during 5 min 142757

After 10 min: recording during 5 min 143258

After 15 min: recording during 5 min 143800

After 20 min: recording during 5 min 144301

After 25 min: recording during 5 min 144803

After 30 min: recording during 5 min 145304

After 35 min: recording during 5 min 145806

After 40 min: recording during 5 min 150307

After 45 min: recording during 5 min 150809

After 50 min: recording during 5 min 151310

After 55 min: recording during 5 min 151812

After 60 min: recording during 5 min 152313

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

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

After 0 min: recording during 5 min 153315

After 5 min: recording during 5 min 153817

After 10 min: recording during 5 min 154318

After 15 min: recording during 5 min 154820

After 20 min: recording during 5 min 155322

After 25 min: recording during 5 min 155823

After 30 min: recording during 5 min 160325

After 35 min: recording during 5 min 160826

After 40 min: recording during 5 min 161328

After 45 min: recording during 5 min 161830

After 50 min: recording during 5 min 162333

After 55 min: recording during 5 min 162835

After 60 min: recording during 5 min 163337

**Other conditions:** Cover blanket, in total darkness (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