**Substance:** NDMC

**Date:** 160318

**Zebrafish DOB:** 160308 (10 dpf)

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

**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)

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

Plate 2:

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

File

**Add NDMC**

**Record during 13x5 min in light/dark**

After 0 min: recording during 5 min (light) 110224

After 5 min: recording during 5 min (light) 110725

After 10 min: recording during 5 min (dark) 111227

After 15 min: recording during 5 min (dark) 111728

After 20 min: recording during 5 min (light) 112230

After 25 min: recording during 5 min (light) 112731

After 30 min: recording during 5 min (dark) 113232

After 35 min: recording during 5 min (dark) 113734

After 40 min: recording during 5 min (light) 114236

After 45 min: recording during 5 min (light) 114738

After 50 min: recording during 5 min (dark) 115239

After 55 min: recording during 5 min (dark) 115741

After 60 min: recording during 5 min (light) 120243

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

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

After 0 min: recording during 5 min 121304

After 5 min: recording during 5 min 121805

After 10 min: recording during 5 min 122307

After 15 min: recording during 5 min 122809

After 20 min: recording during 5 min 123311

After 25 min: recording during 5 min 123812

After 30 min: recording during 5 min 124314

After 35 min: recording during 5 min 124816

After 40 min: recording during 5 min 125318

After 45 min: recording during 5 min 125820

After 50 min: recording during 5 min 130322

After 55 min: recording during 5 min 130824

After 60 min: recording during 5 min 131327

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

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