**Substance:** NDMC

**Date:** 160613

**Zebrafish DOB:** 160603 (10 dpf)

**Aim:** Dose response NDMC/ light

**Volume in wells:** 0.5 ml + 0.5 ml

**Stock NDMC solution:** 50 mM in DMSO

**Dilute to:** **200 µM** (80 µl 50 mM NDMC + 19.920 ml E3)

**100 µM** (5 ml 200 µM NDMC + 5 ml E3)

**50 µM** (2.5 ml 200 µM NDMC + 7.5 ml E3)

**100 µM NDMC:** (0.5 ml 200 µM NDMC) / **50 µM NDMC:** (0.5 ml 100 µM NDMC) / **25 µM NDMC:** (0.5 ml 50 µM NDMC)

**Control:** 0.5 ml 0.4% DMSO/E3 (0.2% in wells)

**Stock Apomorphine solution:** 10 mM in DMSO (300 µl)

**Dilute to: 2500 µM** (300 µl 10 mM Apomorphine + 900 µl E3)

Plate 1:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| A | NDMC 100 µM | NDMC 100 µM | NDMC 100 µM | NDMC 100 µM | NDMC 100 µM | NDMC 100 µM |
| B | NDMC 50 µM | NDMC 50 µM | NDMC 50 µM | NDMC 50 µM | NDMC 50 µM | NDMC 50 µM |
| C | NDMC 25 µM | NDMC 25 µM | NDMC 25 µM | NDMC 25 µM | NDMC 25 µM | NDMC 25 µM |
| D | Control | Control | Control | Control | Control | Control |

Plate 2:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 |
| E | NDMC 100 µM | NDMC 100 µM | NDMC 100 µM | NDMC 100 µM | NDMC 100 µM | NDMC 100 µM |
| F | NDMC 50 µM | NDMC 50 µM | NDMC 50 µM | NDMC 50 µM | NDMC 50 µM | NDMC 50 µM |
| G | NDMC 25 µM | NDMC 25 µM | NDMC 25 µM | NDMC 25 µM | NDMC 25 µM | NDMC 25 µM |
| H | Control | Control | Control | Control | Control | Control |

File

**Add NDMC**

**Record during 13x5 min in light**

After 0 min: recording during 5 min 133307

After 5 min: recording during 5 min 133809

After 10 min: recording during 5 min 134317

After 15 min: recording during 5 min 134831

After 20 min: recording during 5 min 135340

After 25 min: recording during 5 min 135848

After 30 min: recording during 5 min 140358

After 35 min: recording during 5 min 140906

After 40 min: recording during 5 min 141419

After 45 min: recording during 5 min 141931

After 50 min: recording during 5 min 142445

After 55 min: recording during 5 min 142959

After 60 min: recording during 5 min 143513

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

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

After 0 min: recording during 5 min 144420

After 5 min: recording during 5 min 144934

After 10 min: recording during 5 min 145447

After 15 min: recording during 5 min 150001

After 20 min: recording during 5 min 150515

After 25 min: recording during 5 min 151026

After 30 min: recording during 5 min 151541

After 35 min: recording during 5 min 152056

After 40 min: recording during 5 min 152611

After 45 min: recording during 5 min 153126

After 50 min: recording during 5 min 153641

After 55 min: recording during 5 min 154156

After 60 min: recording during 5 min 154713

**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