**Substance:** NDMC

**Date:** 160711

**Zebrafish DOB:** 160701 (10 dpf)

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

**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)

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

Plate 1:

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

Plate 2:

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

File

**Add NDMC**

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

After 0 min: recording during 5 min (light)

After 5 min: recording during 5 min (light)

After 10 min: recording during 5 min (dark)

After 15 min: recording during 5 min (dark)

After 20 min: recording during 5 min (light)

After 25 min: recording during 5 min (light)

After 30 min: recording during 5 min (dark)

After 35 min: recording during 5 min (dark)

After 40 min: recording during 5 min (light)

After 45 min: recording during 5 min (light)

After 50 min: recording during 5 min (dark)

After 55 min: recording during 5 min (dark)

After 60 min: recording during 5 min (light)

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

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

After 0 min: recording during 5 min

After 5 min: recording during 5 min

After 10 min: recording during 5 min

After 15 min: recording during 5 min

After 20 min: recording during 5 min

After 25 min: recording during 5 min

After 30 min: recording during 5 min

After 35 min: recording during 5 min

After 40 min: recording during 5 min

After 45 min: recording during 5 min

After 50 min: recording during 5 min

After 55 min: recording during 5 min

After 60 min: recording during 5 min

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

Exposure: 1340 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