**Substance:** Clozapine

**Date:** 160401

**Zebrafish DOB:** 160322(10 dpf)

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

**Volume in wells:** 1 ml + 20 µl

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

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

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

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

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

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

Plate 2:

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

File

**Add Clozapine**

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

After 0 min: recording during 5 min (light) 111736

After 5 min: recording during 5 min (light) 112237

After 10 min: recording during 5 min (dark) 112739

After 15 min: recording during 5 min (dark) 113240

After 20 min: recording during 5 min (light) 113742

After 25 min: recording during 5 min (light) 114243

After 30 min: recording during 5 min (dark) 114745

After 35 min: recording during 5 min (dark) 115246

After 40 min: recording during 5 min (light) 115747

After 45 min: recording during 5 min (light) 120249

After 50 min: recording during 5 min (dark) 120750

After 55 min: recording during 5 min (dark) 121252

After 60 min: recording during 5 min (light) 121754

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

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

After 0 min: recording during 5 min 122917

After 5 min: recording during 5 min 123419

After 10 min: recording during 5 min 123929

After 15 min: recording during 5 min 124422

After 20 min: recording during 5 min 124924

After 25 min: recording during 5 min 125425

After 30 min: recording during 5 min 125927

After 35 min: recording during 5 min 130429

After 40 min: recording during 5 min 130930

After 45 min: recording during 5 min 131432

After 50 min: recording during 5 min 131934

After 55 min: recording during 5 min 132436

After 60 min: recording during 5 min 132938

**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