

Mineralocorticoid Receptor Transactivation Assay (MR TA)

The steps of the Mineralocorticoid Receptor Transactivation Assay is described in the figure below. The pre-validation is divided into two steps: a transferability and a second phase on blind-coded substances (30 substances).

A study plan and a report will be required by each lab for each phase.

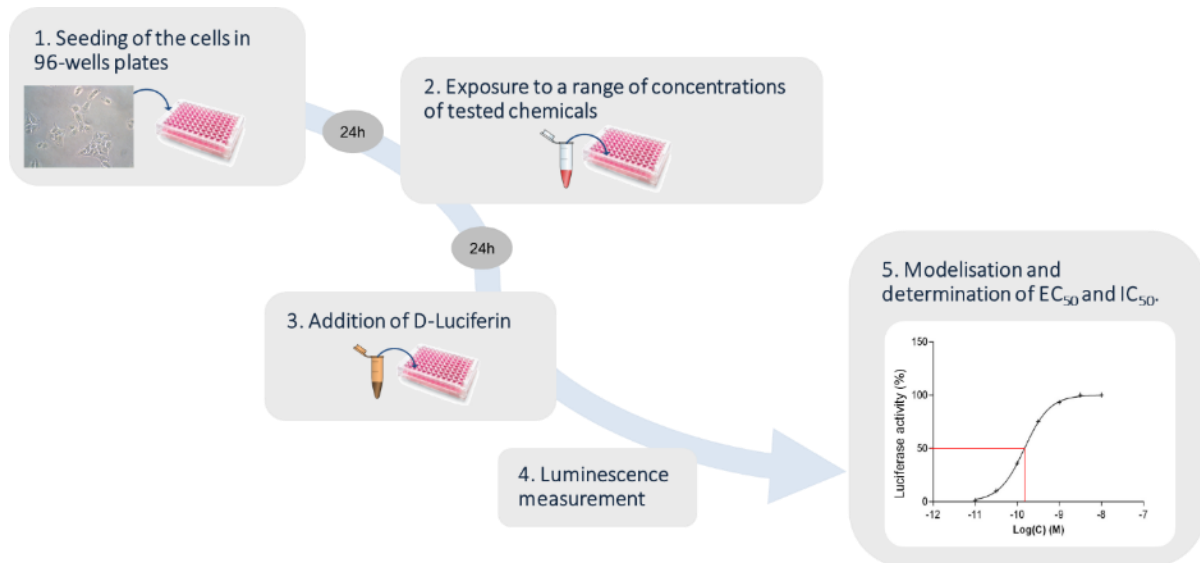


Figure 1: Description of the MR TA assay

Quantification information

Transferability phase

- ✚ **3 modes** will be tested: MR agonist, MR antagonist and MR interference
- ✚ **3 valid runs** (biological replicates) **per test item** are to be performed (a run is an independent experiment characterized by a new set of solutions and controls)
- ✚ 2 substances per plate (several plates are performed in a day)
- ✚ 1 full dose-response curve with control for agonist and antagonist modes for each run
- ✚ 1 positive control + **3 test items** for each mode
- ✚ 7 concentrations per substance will be screened (in quadruplicates) (examples of plate layout in *figure 2*)
- ✚ **Cytotoxicity** is measured for each plate containing test items

Phase II

- + **3 modes** will be tested: MR agonist, MR antagonist and MR interference (only for the chemicals showing an effect with antagonist mode)
- + **3 valid runs** (biological replicates) **per test item** are to be performed (a run is an independent experiment characterized by a new set of solutions and controls)
- + 2 substances per plate (several plates are performed in a day)
- + 1 full dose-response curve with reference control for agonist and antagonist modes for each run
- + 1 positive control + **30 test items** for each mode
- + 7 concentrations per substance will be screened (in quadruplicates) (examples of plate layout in *figure 2*)
- + **Cytotoxicity** is measured for each plate (on the same plate after the luminescence reading)

Quadruplicates

Medium control	Reference control C7	Solvent control	Substance 1 C1	Substance 1 C2	Substance 1 C3	Substance 1 C4	Substance 1 C5	Substance 1 C6	Substance 1 C7	Reference control C7	Medium control
			Substance 2 C1	Substance 2 C2	Substance 2 C3	Substance 2 C4	Substance 2 C5	Substance 2 C6	Substance 2 C7		




Figure 2 : Example of a plate layout (C=concentration)

The offer should include :

- + A contingency plan (including any changes about the workforce, work continuity during holidays...)
- + A description of the quality system set up in the lab