DISPENDIX
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ADP Glo™ assay

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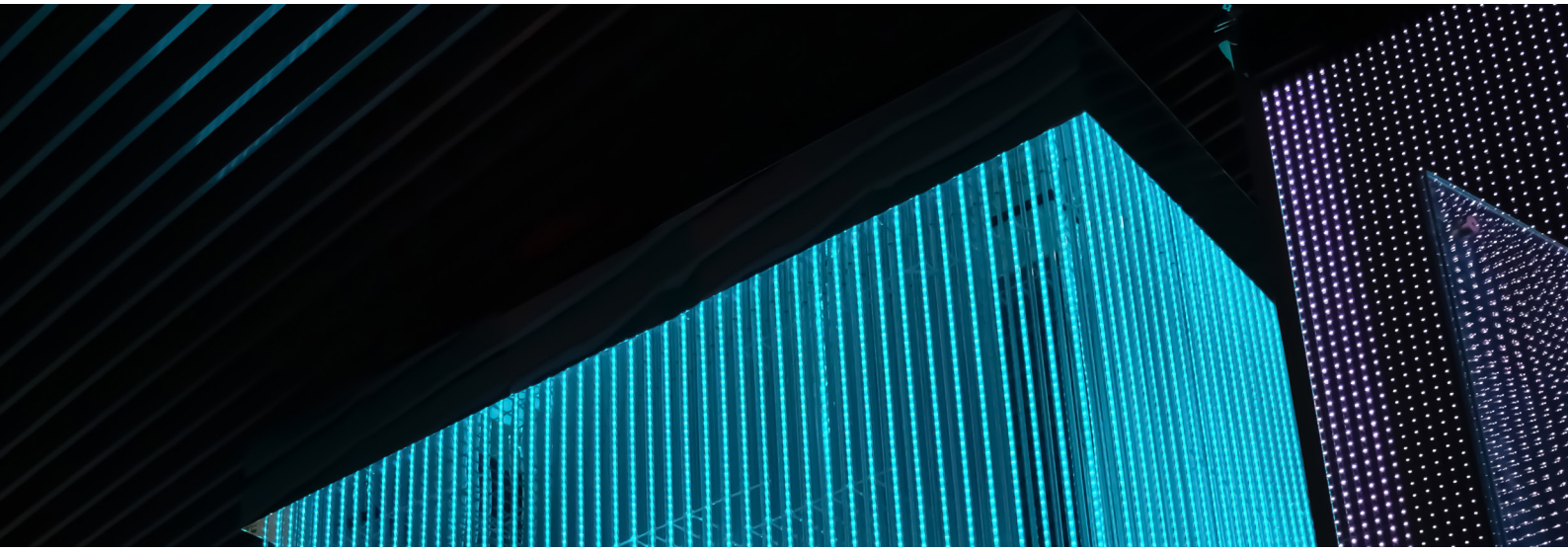

Introduction

ADP Glo™ assay

We use an ADP-Glo™ assay (Promega) (see ***Figure 1***) to test different compounds in dose response and in the meantime look at the substrate dependency of this compound. We work in a relative small assay volume so to make the pipetting more doable it is easy to spot a dose response of the substrate in de assay plate, and add the compound dose response manually or with a robotic system (Fluent).

Materials and methods

We make a dose response of the substrate in a buffer solution. We place the cups in the source plate of the I.DOT and fill them with the dose response of the substrate. We spot this dose response with the I.DOT on the destination plate (see ***Figure 2***). Than the compound dose response is added manually or with a robotic system like the Fluent (TECAN). The reaction is started by dropping ATP using a multidrop, this allows us to work fast, robust and gives the opportunity to drop small volumes.

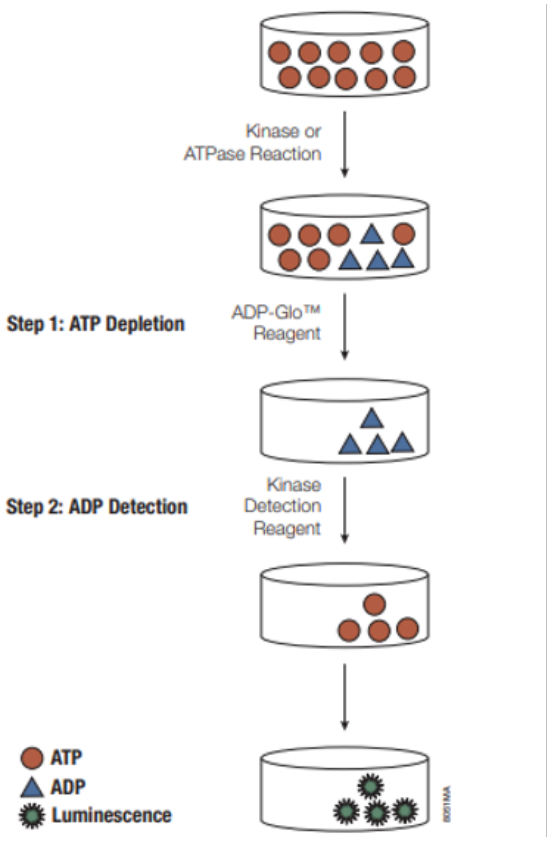

Figure 1. *ADP Glo™ assay principle*

After 30 min at 37ºC the reaction is stopped with ADP Glo™ Stop using a multidrop to deplete not consumed ATP. After 40 min at RT ADP Glo™ Detection is added with a multidrop to the plate to convert ADP to ATP. After 1h at RT we measure Luminescence with an Envision. All the data is plotted with Prism (GraphPad)

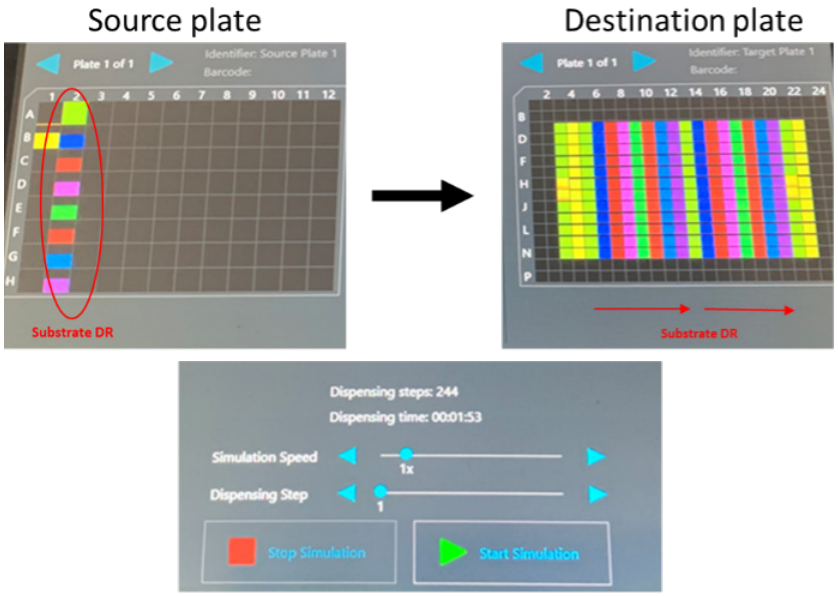

Figure 2. *Layout from the software*

Results

Graphs of the Dose dependency of the Substrate and the shift (Vmax/Km) with different compound concentrations is calculated (see ***Figure 3***).

Conclusions

With the I.DOT it is possible to spot easy and precise the dose response of the substrate. So that we have more volume left to add other components to the reaction. This set up gives us the possibility to get the most out of this experiment. So we can decide how to proceed with this project.

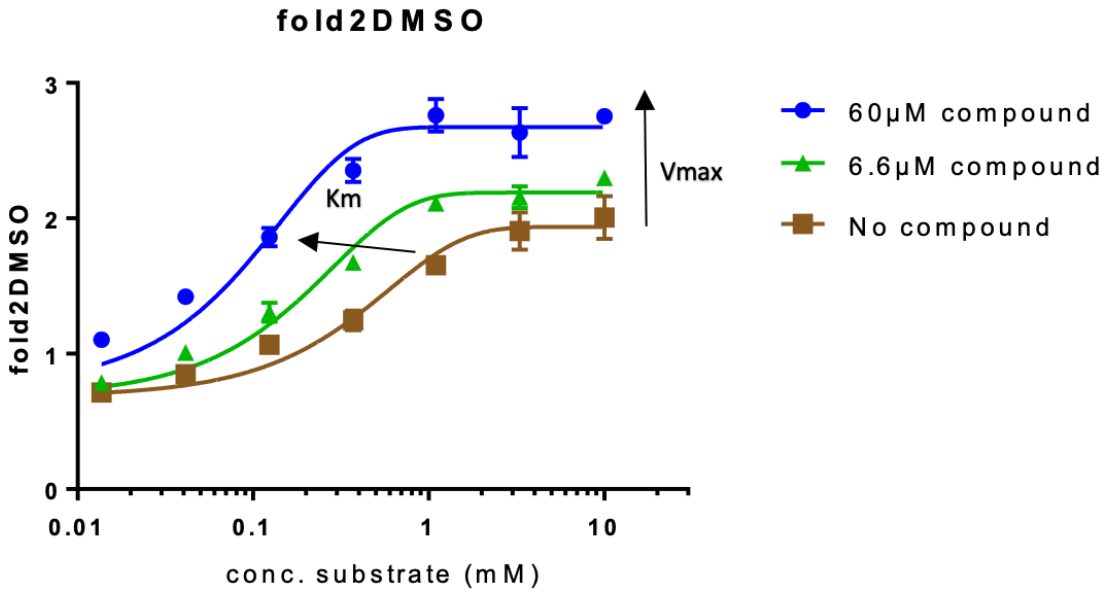

Figure 3. *Graphs from graphpad*

References

1. https://be.promega.com/-/media/files/resources/protocols/technical-manuals/0/adp-glo-kinase-assay-protocol.pdf?rev=42e85ccab87244b395f4ecd1ad8e1616&sc_lang=en

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