A fluorogenic assay for identification of coagulation factor IIa inhibitors-screening for stroke treatment

Easy automation of a biochemical assay on the Fluent™ laboratory automation solution

Introduction
The protease thrombin (Factor IIa) is an important drug discovery target, playing a central role in the blood coagulation and wound healing processes. This role is established by the fact that any perturbation within the blood coagulation system resulting in significant amplification, impairment, acceleration or delay in thrombin generation leads to clinically relevant hemorrhagic or thrombotic events. There are many ongoing efforts in the pharmaceutical industry to identify compounds which can inhibit thrombin activity. Here we present a fluorogenic assay for identification of coagulation factor IIa inhibitors in 384 well plates.

Material and methods
The assay for the identification of the coagulation factor IIa inhibitors was performed on the Fluent 780 assay workstation (Figure 2). 384 well assay plates were automatically retrieved from the Carousel and thrombin was added. After a 10 minute incubation step at room temperature in the Carousel, the substrate was added and the plate incubated at room temperature for further 30 minutes (Figure 1). Finally, the fluorescence signal in the wells was detected using the fully integrated Infinite® M1000 PRO reader and the percentage of thrombin inhibition as well as Z' were determined.

Workflow

Results
Very good reproducibility (Figure 3 and 5) and Heatmaps (Figure 4).

Summary
This application note demonstrates that automation of biochemical assays such as the thrombin assay, in 384-well format can be performed in a straightforward manner using Tecan’s, Fluent laboratory automation solution. The 20 plate assay was complete in 4.5 hours, generating 7,680 individual data points. The large deck capacity and the multi-tasking of the eight-channel Flexible Channel Arm, the Multiple Channel Arm and the Robotic Gripper Arm allow the full assay to be completed in such a short time and without human intervention. Full details, see application note entitled ‘A fluorogenic assay for identification of coagulation factor IIa inhibitors-screening for stroke treatment’.

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Thrombin assay plate layout

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